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Association Between Dairy Intake And Fracture In An Australian Based Cohort Of Women

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Abstract

Objective: Given the inconsistent evidence on dairy consumption and risk of fracture, we assessed the association between milk/total dairy consumption and fracture in women from the Geelong Osteoporosis Study (GOS).

Methods: Women aged ≥50yr (n= 833) were followed from baseline (1993-1997) to date of first fracture, death, or 31 December 2017, whichever occurred first. Milk/dairy consumption was assessed by self-report. Fractures were confirmed radiologically. Total dairy consumption was calculated by considering milk, cheese, yogurt and ice-cream intake. Multivariable adjusted Cox proportional hazard models, adjusted for potential confounders, were used to determine associations between milk/total dairy consumption and major osteoporotic fracture (MOF). Associations between milk/total dairy consumption and serum high sensitivity C-reactive protein (hsCRP), C-terminal telopeptide (CTx) and procollagen type 1 N-terminal propeptide (P1NP) were investigated using multivariable linear regression.

Results: During follow-up (11,507 person-years), 206 women had a MOF. The multivariable adjusted hazard ratio (HR) for women who consumed >500 mL/d of milk (1.15, 95% CI 0.75,1.75, p=0.53) was not significantly higher compared to women who consumed <250 mL/d of milk. The multivariable adjusted HR for fractures in women consuming ≥ 800 g/d total dairy (1.70, 95% CI 1.00, 2.93, P=0.05) was higher compared to women who consumed 200-399 g/d total dairy and reached marginal significance. Milk consumption was inversely associated with serum hsCRP and CTx but total dairy consumption was not associated with these serum markers.

Conclusion: Increased milk consumption is not associated with increased risk for MOF, whereas increased total dairy consumption is associated with an increased risk for MOF in older women.

Key words: Fractures, Milk, Osteoporosis, Dairy, Inflammation

Strengths and limitations of the study

- ⇒ Although this study contained a modest sample size, it replicated the findings of previous studies.
- ⇒ The likelihood of bias is minimal due to random sample selection from the general population.
- ⇒ The prospective study design strengthens the outcomes of the study despite methodological inconsistencies in capturing dietary data.
- ⇒ As data for total dairy consumption were available at baseline only, we cannot account for dietary changes during follow-up and this limits the interpretation of the longitudinal analysis of the association between total dairy consumption and the risk for MOF
- ⇒ The conclusions of this study cannot be generalised as this study was focused on a cohort of women.

Introduction

Fractures can occur as a result of low bone mass and impaired bone micro-architecture due to osteoporosis, a chronic multifactorial disease (1, 2). Apart from genetics (3, 4), age (5, 6), lifestyle habits (2) and sex (7), nutrition plays a substantial role in the aetiology of osteoporosis (8, 9). Adequate calcium and protein intakes are necessary in order to maintain skeletal integrity and strength (10, 11). Milk/dairy products are key components in the western diet and contain a myriad of nutritional components (calcium, vitamins and proteins) and a majority of an individual's dietary calcium needs are fulfilled by intake of dairy products (12, 13). Additionally, milk/dairy products have been widely recommended to osteoporosis patients by clinicians and healthcare professionals considering the beneficial effects associated with dairy consumption (14, 15).

However, data regarding milk consumption as a strategy for fracture prevention has shown inconclusive results. Findings from large Swedish cohorts reported that women who consumed three or more glasses of milk per day had higher risk for any fractures while fermented dairy consumption was inversly associated with fractures (16). However, Feskanich *et al.* have shown in two large US cohorts that each serving of milk per day was associated with an 8% reduction of risk for hip fracture, whereas total dairy intake was associated with a 6% reduction of risk for hip fractures in men and women combined (17). A meta-analysis published in 2010 showed no overall association between milk consumption and hip fractures in women (18). Although increased milk intake appeared to be protective for men in this study, firm conclusions cannot be drawn due to the limited data points and the authors noted that further studies were needed (18). Moreover, the most recently published meta-analysis (2018), which included 18 observational studies, failed to show that higher milk intakes were associated with

fractures in both sexes combined (19). Similarly, Holvik *et al*. found no association between increased milk intake and risk for hip fractures in Norwegian women and men (20).

Given the burden of osteoporosis (21) and the inconclusive nature of the results in the field, we aimed to assess the association between milk consumption and risk for major osteoporotic fracture (MOF) in a large representative sample of Australian women. In addition, we also assessed the association between total dairy consumption and MOF. We hypothesised that increased milk and total dairy consumption may be associated with increased risk for MOF and also investigated potential mechanisms by which increased milk/dairy may mediate the risk for MOF.

Methods

Patient and Public involvement

Patients were not involved in the planning and design in the study.

Study Population

This study used data from the Geelong Osteoporosis Study (GOS), a large population-based cohort study based in south-eastern Australia. Inclusion criteria were: living in the Barwon Statistical Division (BSD) for > 6 months and able to provide written informed consent. Women in the BSD were selected at random from the electoral roll during the years 1993-1997 to participate in the study (22). An age-stratified sample of 1,494 women was enrolled in the study with a participation of 77.1%. Subsequent assessments for these women commenced in 1995, 1998, 2000, 2002, and 2004, referred to as 2-year, 4-year, 6-year, 8-year, and 10-year follow-up phases. The 2-year follow-up encompassed 1,180 women from baseline representing retention of 85%. Subsequently, the 4-year and 6-year follow-up reported retention of 70% and

86%, respectively. The 10-year follow-up included 881 eligible participants giving a retention of 83%. Women aged ≥50yr at baseline were included in this study, resulting in a total of 833 women for this analysis. Study participants provided written informed consent. The study was approved by the Human Research Ethics Committee at Barwon Health.

Outcome Measures

Radiological reports were used to identify and confirm post-baseline incident fractures using a method that has been validated for use in the study region; only MOFs during the follow-up period were included (23, 24). MOFs were defined as fractures at the hip, forearm, clinical spine and proximal humerus, according to the fracture risk assessment tool (FRAX) developed by the University of Sheffield for clinical use (25). Pathological and high trauma fractures were excluded.

Dairy consumption and diet

At baseline and 6-year follow-up, dietary information was documented by a self-reported questionnaire that contained questions on 35 foods and beverages on average. Participants were asked questions about the usual (habitual) type of milk consumed (whole, reduced fat, calcium fortified, soy, goat's milk, butter milk, and evaporated) and the quantity consumed each day. In the questionnaire, it was stated that one cup of milk is considered equivalent to 250 mL. Therefore, participants chose the type and quantity of milk consumed from any pre-determined milk categories and only cow's milk was considered (none, < 125 mL (< ½ cup), 125 -249 mL (½-<1 cup), 250-499 mL (1-<2 cups), 500- 999 mL (2-<4 cups), ≥ 1000 mL (≥ 4 cups) per day). The lowest response categories <125 mL/d, 124-249 mL/d were collapsed into one category indicating "< 250 mL/d" and the highest response categories, 500- 999 mL/d, ≥ 1000 mL were combined into one category indicating "> 500 mL/d"; this was due to low proportions

responding to the lower and higher categories and for the compatibility with the 10-year follow-up data. Information on other dairy products such as cheese, yogurt and ice-cream consumption were also documented using this self-reported questionnaire. Participants were specifically asked about different types of cheese they consumed on a weekly basis including: hard cheese (servings/week; 1 serving = 16 g); soft cheese (servings/week; 1 serving= 20 g); and fruche (servings/week; 1 serving = 100 g). Fruche is a form of soft cheese (fromage frais) and thus was categorised as cheese. Total cheese consumption was converted to grams consumed per day (g/d). Yogurt (servings/week; 1 serving= 200 g) and ice-cream consumption (servings/week; 1 serving = 27 g) were reported as servings per week and this was converted to grams consumed per day. Daily total dairy consumption was calculated by combining values for cow's milk, all forms of cheese, yogurt and ice-cream consumed and was expressed in grams per day. Information on milk/dairy consumption was collected at the 10-year follow-up using a validated food frequency questionnaire. The Cancer Council Victoria Dietary Questionnaire captures information on 74 foods and six alcoholic beverages over the previous 12 months and is validated for assessing habitual dietary intake in Australian women (26). Participants were interrogated on their usual type (none, full cream, reduced fat, skim, and soy milk) and quantity of milk consumed on a daily basis. Participants were advised that 1 cup of milk is equivalent to 250 mL of milk. Furthermore, participants indicated their daily milk intake by selecting from pre-determined categories of milk intakes and only cow's milk was considered (none, < 250 mL (<1cup), 250- 499 mL (1-<2 cups), 500- 750 mL (2-3 cups) and > 750 mL (> 3 cups) per day). The highest response categories, 500-750 mL/d, > 750 mL/d were combined as to one category indicating "> 500 mL/d"; this was due to low proportions responding to the higher categories. This questionnaire also captured information on cheese, yogurt and ice-cream intake of participants.

A separate calcium-specific dietary questionnaire was used to capture information on dietary calcium intake. This questionnaire included information on a range of common calcium-dense food sources, which allowed calculation of dietary calcium intakes in mg per day (mg/d) and validated against 4-day weighed food intakes (13). Dietary calcium intake was categorised into two strata (< 1000 mg/d, ≥ 1000 mg/d).

Other information and potential confounders

All measurements were assessed at the baseline visit. Weight and height were recorded to the nearest 0.1 kg and 0.1 cm respectively and body mass index (BMI) calculated as weight/height^2 (kg/m^2). Dual-energy x-ray absorptiometry (DXA; Lunar DPX-L; Lunar, Madison, WI) was performed to evaluate bone mineral density (BMD; g/cm^2) at the femoral neck, and whole-body fat (kg), and 'lean' mass (kg) which represents the water and protein content in muscle, skin, connective tissue and lean component in adipose tissue.

Self-report questionnaires were used to obtain information on mobility, physical activity levels, smoking status, medications, prior falls, and fractures. Participants were asked to select their mobility level from pre-determined 7-point scale (very active, active, sedentary, limited, inactive, chair or bed ridden, bedfast - examples were given in the questionnaire to assist the participant to choose the most suitable option). These categories were further condensed to two groups, highly active and less active, for the purpose of this analysis. Physical activity level was also assessed from questions regarding work/home and recreational/sports, on a 3 point-scale which provided options for participants to select from moderate, hard and very hard. Participants were also asked to enter the time spent on each activity level on a weekly basis.

Information on current smoking status was categorised as smoking or non-smoking. Use of medications that positively or negatively influence bone included bisphosphonates, anabolic

therapies, hormonal replacement therapies (HT), and oral glucocorticoids. Participants were asked to list the use of supplements and this information was used to assess the calcium and vitamin D supplementation usage. Use of supplementary calcium and vitamin D were documented at baseline, 6 yr and 10 yr follow-up.

The definition of falls (when you suddenly find yourself on the ground, without intending to get there, after you were in either a lying, sitting or standing position) was explicit in the questionnaire and asked participants whether or not they experienced a similar scenario over the past 12 months. Information regarding previous fractures and cancer diagnoses was also captured by self-reported questionnaires. An automated device (Takeda Medical UA-751) was used to measure blood pressure in a sitting position. Women were considered hypertensive if they had a systolic blood pressure over 140 mmHg and/or a diastolic pressure above 90 mmHg and/or use of antihypertensive medication in the presence of self-reported hypertension. Women were identified as having diabetes if they had a fasting plasma glucose ≥ 7.0 mmol/L (126 mg/dL), self-reported diabetes and/or use of antihyperglycaemic agents.

Information pertinent to educational qualifications were gathered on a 7-point scale: never attended school, primary school, some secondary school, completed secondary school, post-secondary qualifications, university or other tertiary qualifications, and can't remember. These categories were compressed to education received for less than 12 years or more than 12 years for the purpose of this analysis. Information on marital status was dichotomised as living alone or living with a partner. The socio-economic status of the cohort participants was measured by the Index of Relative Socioeconomic Disadvantage (IRSD), an area-based index that measures relative disadvantage of socio-economic status. This tool imputes a span of information on economic and social conditions of people and household within an area and is represented in quintiles. The most disadvantaged category is indexed by quintile 1 (27).

Biomarkers

At baseline, venous blood was collected after an overnight fast and stored at -80 ^{0}C until batch analysis. Markers of bone turnover, serum C-terminal telopeptide (CTx), a marker of bone resorption, and serum procollagen type 1 N-terminal propeptide (P1NP), a marker of bone formation, were analysed from the blood samples. In addition, high sensitivity serum C-reactive protein (hsCRP), a marker of systemic inflammation, was determined from the blood samples. Serum hsCRP was measured by the Roche immunoturbidometric 'CRP" and 'C-reactive protein (latex) high sensitivity methods. Details of these analytical methods have been described elsewhere (28, 29).

Statistical analysis

Characteristics of participants were described by mean (± SD) or median (IQR) or relative frequencies (%) stratified by milk consumption categories (no milk, <250 mL/d, 250-500 mL/d, > 500 mL/d). Participant characteristics across categories of milk consumption were compared using one-way ANOVA or Kruskal-Wallis-H test for continuous data and Chi-square test (or Fisher's exact test) for categorical data. The null hypothesis was rejected at an α level of 0.05 and a post hoc multiple comparison was performed using Bonferroni corrections.

Cohort participants were followed from their baseline appointment to date of first fracture, death, or 31 December 2017. Cox proportional hazard regression was used to estimate age adjusted hazard ratio (HR) and their 95% confidence intervals for categories of milk consumption (no milk, < 250 mL/d, 250-500 mL/d, > 500 mL/d). Further, this model was adjusted for oral glucocorticoid use, HT use and prior fractures. The exposure variable and risk factors were time updated at follow-up visits whenever data were available. Information on

milk consumption, oral glucocorticoid use, HT use was time up-dated in 6 and 10-year follow-up and information on prior fractures was only included at baseline.

In addition, a Cox proportional hazard regression was also used to estimate the age adjusted HR and their 95% confidence intervals for total dairy consumption categories (< 200 g/d, 200-399 g/d, 400-799 g/d, ≥ 800 g/d). Further, this model was adjusted for oral glucocorticoid use, HT use, and prior fractures. For this analysis we only considered the baseline values for total dairy consumption due to the inconsistent methods in capturing information on other dairy products. However, we time updated oral glucocorticoid use, HT use, and prior fractures at follow-up visits whenever data were available. The proportional hazard assumptions were confirmed graphically by log(-log(survival)) plots for both daily milk and total dairy consumption. Time to first fracture (survival) curves were illustrated using Kaplan-Meier estimator of the survival function using product limit estimator.

Associations between milk/total dairy consumption and serum markers of inflammation (hsCRP) and bone turnover (CTx, PINP) were also assessed using multivariable linear regression models at baseline with potential confounders. Serum markers of inflammation and bone turnover were log transformed due to the skewed nature of data. For all analyses, STATA 15 and SPSS 25 was used.

Results

Descriptive characteristics of the cohort stratified by milk consumption categories are presented in Table 1. Of 833 women, 8.4% (*n*=70) did not consume milk and 47.2% (*n*=393), 34.3% (*n*=286) and 10.0% (*n*=84) consumed < 250 mL/d, 250-500 mL/d and > 500 mL/d of milk, respectively. There was no difference observed in women's median age among the four-milk consumption categories. Women who consumed >500 mL/d of milk reported the highest

cheese intake. The group that consumed < 250 mL/d of milk had the highest proportion of women reporting < 1000 mg/d of dietary calcium intake. On the other hand, the group that consumed > 500 mL/d of milk had the highest proportion of women reporting ≥1000 mg/d of dietary calcium intake (Table 1). The proportion of women consuming supplementary calcium and vitamin D was high among the non-milk consumers. There were no differences detected for other parameters across the four-milk consuming groups.

During 11,507-person years of follow-up, 206 women sustained a MOF (spine=96; humerus=14; wrist=51; hip=45) and 503 women died. Women who consumed no milk reported the highest fracture rate (Table 2) and the fracture survival probability curve also showed that women who consumed no milk had the lowest survival probability for fractures (Figure 1). The unadjusted (1.28, 95% CI 0.84,1.96, *P*=0.25), age adjusted (1.23, 95% CI 0.80,1.88, *P*=0.34), and multivariable adjusted (1.15, 95% CI 0.75,1.75, *p*=0.53) HR for MOF were not higher in women who consumed > 500 mL/d of milk compared to women who consumed < 250 mL/d of milk. However, women who reported no milk consumption showed marginally significant higher age adjusted (1.54, 95% CI 0.98, 2.44, P= 0.06) and multivariable adjusted (1.56, 95% CI 0.99, 2.46, *P*=0.06) HR for MOF compared to women who consumed < 250 mL/d of milk.

When total dairy consumption was considered, women who consumed more than ≥ 800 g/d demonstrated the highest fracture rate (Table 3). This was also confirmed by the fracture survival probability curve, which indicated the lowest survival probability for fractures in women who consumed ≥ 800 g/d total dairy (Figure 2). Consistently, women who consumed ≥ 800 g/d total dairy showed higher age adjusted (2.01, 95%CI 1.88, 3.44, *P*=0.01) and multivariable adjusted (1.70, 95% CI 1.00, 2.93, *P*=0.05) HR for MOF compared to women who consumed 200-399 g/d of total dairy (Table 3).

An inverse association was observed between milk consumption and serum markers of inflammation (hsCRP) and serum markers of bone resorption (CTx); women who consumed > 500 mL/d of milk had the lowest concentrations of serum hsCRP (-0.45; 95%CI: -0.82, -0.07; *P*= 0.02) and serum CTx (-0.25; 95% CI: -0.48, -0.02; *P*= 0.03) (Table 4). No association was found between milk consumption and serum marker of bone formation (PINP). Moreover, there was no association found between total dairy consumption categories and serum hsCRP, CTx and PINP (Table 4).

Discussion

In our study of older Australian women, we detected no significant association between higher milk consumption (> 500 mL/d) and increased risk for MOF. However, we found that zero milk consumption was associated with increased risk for MOF. In addition, our study results demonstrated that consuming higher amount of total dairy (> 800 g/d) was associated with an increased risk for MOF.

Acquiring the daily recommend calcium through diet is considered the easiest and safest lifestyle modification that could be achieved as a part of prevention and management of osteoporosis. Milk/dairy products are considered the ideal source of calcium that, consumed in recommended quantities, may approximately satisfy the daily calcium requirements (30, 31). In general, 1200 mg/d of calcium is recommended for women aged > 50yr (32) and potentially four serves of milk (1 serve = 250 mL = 300 mg of calcium) can cover this need. Our study results revealed that consuming no milk was associated with increased risk of MOF.

Some components in milk such as D-galactose, a milk sugar (33) and A1-beta-casein, a mutated form of milk casein (34) are believed to possibly mediate the unfavourable consequences associated with milk consumption. A study published in 2014 by a group of Swedish

researchers showed that women who consumed more than 3 glasses of milk compared to 1 glass of milk per day had higher risk for any fractures and mortality (16). The authors speculated that increased milk intake may be deleterious to bone due to the D-galactose content in milk. D-galactose has proven to be involved in the ageing process in mice that encompassed series of events such as oxidative stress and chronic inflammation (35). Concordantly, a positive correlation between milk consumption and both oxidative stress marker in urine (8-iso-PGF2α) and inflammatory marker in serum (IL-6) was detected in the Swedish cohort (16). Hence, those findings offered support to the hypothesis that increased milk intakes are deleterious to bone due to the D-galactose in milk (16). However, many other studies (18-20) including our study did not find any evidence to show that increased milk consumption is associated with fractures. Sweden is one of the countries that reports the highest consumption of A1-beta-casein in form of milk/dairy products (36). This protein type in milk may serve as another possible explanation for the positive association observed between higher milk intake and fracture risk in the Swedish mammography cohort.

A1 beta-casein is a mutated form of beta-casein protein, originally produced from A2 beta-casein due to a gene mutation occurred in the European Holstein herds ten-thousand years ago and conventional milk often contains a mixture of A1 and A2 beta-casein (37). The enzymatic digestion of A1 beta-casein generates beta-casomorphin-7 (BCM-7), a bioactive peptide with claimed opioid properties (38). Existing epidemiological data show that some negative health consequences associated with milk consumption may be due to the A1 beta-casein fraction in milk. Country level studies in past have shown a positive correlation between A1 beta-casein consumption and ischemic heart diseases (36, 39, 40) and type I diabetes (41, 42). In addition, evidence from animal studies demonstrates the potential of BCM-7 to induce inflammation

(43). However, the perception on how A1 beta-casein and BCM-7 may trigger inflammation in humans is still at its early stage.

Most importantly, other milk-derived products such as yogurt, cheese, also contain elements from milk (D-galactose, A1 beta-casein), which are considered detrimental. Fermented dairy contains higher concentration of D-galactose (44-46) and Noni *et al.* (47) showed that fermented dairy such as cheese and yogurt produces significant amount of BCM-7 when subjected to proteolytic enzymatic digestion. Therefore, we assessed the association between total dairy consumption (milk, cheese, yogurt and ice-cream) and MOF. Here, we found that women who consumed > 800 g/d of total dairy showed higher risk for MOF compared to women consuming moderate levels.

The present study hypothesised two potential mechanisms to elucidate how increased milk/dairy consumption may instigate fractures. The first is the inflammation concept: augmented systemic inflammation impacts bone homeostasis negatively and lead to increased risk of fragile bones and fractures (29, 48). Therefore, we hypothesised that increased milk/total dairy consumption may augment systemic inflammation considering the pro-inflammatory elements of milk and studied the association between milk consumption categories and serum marker of inflammation (hsCRP). Serum hsCRP is deemed a sensitive marker of systematic inflammation and higher concentration of serum hsCRP has been detected in inflammatory diseases (49). However, in this study, the lowest serum hsCRP concentration was detected in women who consumed > 500 mL/d of milk. Our findings did not support our hypothesis and they were corroborated by other literature that showed decreased CRP levels with increased milk/dairy intake (50). Also, we did not find any association between total dairy consumption and serum hsCRP.

The second concept is pertinent to altered bone metabolism: lower osteoblastic activity may increase bone fragility and increase the propensity for fractures. The opioid peptide, BCM-7, yielded from A1 beta-casein fraction in milk has demonstrated properties of morphine (40, 51) and evidence shows that morphine reduces osteoblastic activity and is also associated with increased risk for fracture (52, 53). Therefore, we hypothesised that increased milk/total dairy consumption may alter bone metabolism unfavourably due to the opioid peptides yielded during digestion. However, women consuming >500 mL/d of milk had the lowest concentrations of serum marker of bone resorption (CTx), where no clear patterns of associations were found between milk consumption and serum marker of bone formation (PINP). Moreover, there was no association detected between milk/total dairy consumption and serum CTx and PINP.

Our study has several strengths. Although we only considered individuals aged ≥50 years for the purpose of our analysis, the GOS comprises a large sample of randomly-selected participants from the full adult age range recruited from electoral rolls. As voting is compulsory in Australia, the electoral rolls are a comprehensive register of all adults within Australia, and would be representative of the general population, thus the likelihood of bias is minimal. Additionally, as the GOS is a cohort study, we performed longitudinal analyses that incorporated a long follow-up time with a median of 14.26 years.

Data on the main exposure variable and other variables were updated several times during the period of follow-up, which enhanced the robustness of our analyses. Cognisant that osteoporosis is a multifactorial disease, we included many possible potential confounders (age, oral glucocorticoids, HT, and past fractures) in the analysis. Also, we used an objective method of ascertaining/confirming incident fractures from radiological reports rather than relying on self-reported information.

However, our study did have some limitations. Most of the exposure data gathered were self-reported, which may be subject to recall bias and inaccurate reporting with both random and systematic bias possible. We were unable to update total dairy consumption in the Cox regression analysis, as dietary information was not collected consistently across all follow-up visits and we thus performed an analysis using the baseline data only. This might have led to unaccounted changes in exposure status that may have occurred during the period of follow-up. Future studies would benefit from using standard/validated and consistent methodology/questionnaire throughout all the follow-up visits to better capture dietary information. The study sample size was modest. A post-hoc power calculation showed that based on annual fracture rate of 14.10 per 1,000 in the reference group (<250 mL/d milk consumption) the minimum detectable effect size (i.e. RR) ranged from 1.5 to 1.9, which was bigger than observed risk ratios from unadjusted and adjusted Cox models.

In addition, some participants were lost to follow-up during the study, which prevented time-dependent updates on their information. As with all observational/follow-up studies, attrition is unavoidable and implementing strategies to enhance retention (e.g., provision of incentives) may aid in increasing retention rates. In the interim, there may have been other unrecognised confounding in our study. We also did not record changes in the type of milk consumed over the duration of follow-up and could not differentiate between exposure to conventional dairy products or A2 milk products; thus, we were not able to investigate particular milk proteins as mediating potential negative effects associated with milk consumption.

Conclusion

Taken together, our study results suggest that increased milk consumption is not associated with increased risk for MOF; however, zero milk consumption appears to be associated with an increased risk for MOF. Also, increased consumption of total dairy (milk, yogurt, cheese and ice-cream) may increase the risk for MOF, indicating a negative influence on bone health. Further studies are warranted to identify optimal levels of milk and total dairy consumption ranges and the potential mechanisms by which total dairy consumption may influence the risk for fracture.

Contribution: HA, KLH-K, MM, FNJ, JAP contributed to the interpretation of data, and critical appraisal of the manuscript and HA constructed the manuscript.

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Patient consent for publication: not required.

Ethical approval: The study was approved by the Human Research Ethics Committee at Barwon Health.

Data sharing statement: Data for this study will be available upon request. Request can be sent to gos@barwonhealth.org.au.

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TABLE 1

Baseline characteristics of participants[1]

	No milk	< 250 ml/d	250-500 ml/d	> 500 ml/d	P value
Number of women	70	393	286	84	
Age at entry, yr	68.2 (58.2-77.6)	69.1 (59.2-80.3)	71.4 (60.5-80.4)	71.7 (64.2-80.4)	0.19
Body mass index, kg/m^2	25.1 (22.1-28.6)	26.8 (24.1-30.3)	25.9 (23.5-29.9)	25.3 (23.2-28.9)	0.02
Yogurt, g/d	0.0 (0.0-57.1)	0.0 (0.0-57.1)	3.6 (0.0-57.1)	0.0 (0.0-85.7)	0.48
Cheese, g/d	9.1 (3.4-22.9)	9.1 (4.6-16.0)	11.0 (4.6-22.9)	**13.7 (6.9-25.1)	0.04
Ice-cream, g/d	0.0 (0.0-11.6)	0.0 (0.0-7.7)	0.0 (0.0-7.7)	0.0 (0.0-11.6)	0.97
Bone mineral density, g/cm^2	0.792 ± 0.163	0.830 ± 0.156	0.832 ± 0.146	0.808 ± 0.161	0.17
Whole-body fat, kg	24.1 (18.9-32.2)	27.6 (20.7-34.0)	25.7 (20.47-32.75)	24.6 (19.5-29.0)	0.02
Lean mass, kg	36.3 ± 4.8	37.3 ± 4.7	37.3 ± 4.6	36.9 ± 4.1	0.44
Dietary calcium, *n* (%)					0.01
<1000 mg/d	65 (93)	**386 (98)	246 (86)	1(1)	
≥1000 mg/d	5 (7)	4 (1)	39 (13)	**82 (98)	
Falls in the past, *n* (%)	14 (20)	73 (19)	63 (20)	21 (25)	0.52

Fractures in the past, *n* (%)	24 (34)	146 (37)	93 (33)	39 (46)	0.13
Incident cancer, *n* (%)	7 (10)	58 (15)	37 (13)	14 (17)	0.61
Diabetes, *n* (%)	6 (9)	30 (8)	22 (8)	11 (13)	0.74
Hypertension, *n* (%)	47 (67)	242 (62)	172 (60)	51 (61)	0.76
Smoking, *n* (%)					0.18
Smokers	64 (91)	348 (89)	267 (93)	78 (93)	
Non-smokers	6 (9)	45 (11)	19 (7)	6 (7)	
Mobility, *n* (%)					0.86
Highly active	38 (54)	192 (49)	142 (50)	43 (51)	
Less active	32 (46)	201 (51)	144 (50)	41 (49)	
Supplemental calcium, *n* (%)	**16 (22)	49 (12)	34 (12)	17 (20)	0.03
Supplemental vitamin D, *n* (%)	**15 (21)	45 (11)	25 (9)	15 (18)	0.01
Bisphosphonates, *n* (%)	1 (1)	1 (0)	6 (2)	0 (0)	0.07
Anabolic therapies, *n* (%)	0 (0)	0 (0)	0 (0)	0 (0)	
HT, *n* (%)	10 (14)	70 (18)	38 (13)	8 (10)	0.18
Oral glucocorticoids, *n* (%)	2 (3)	9 (3)	5 (2)	6 (7)	0.07

IRSD *n* (%) / quintile					0.89
1	11(15)	81(21)	48 (17)	15 (18)	
2	18 (26)	81(21)	63 (22)	19 (24)	
3	16 (23)	97 (25)	58 (20)	20 (24)	
4	12 (17)	58 (14)	55 (19)	14 (17)	
5	13 (19)	76 (19)	62 (22)	16 (19)	
Education, *n* (%)					0.57
<12 years	63 (90)	342 (87)	248 (87)	69 (82)	
≥12 years	7 (10)	46 (11)	37 (13)	14 (17)	
Marital status, *n* (%)					0.16
Living with partner	28 (40)	162 (41)	137 (48)	43 (51)	
Living alone	42(60)	231(59)	149 (52)	41 (49)	

[1] Data reported as mean± SD, median (IQR) or n (%); Milk comprises skim, low fat, full fat with a serving size of 1 cup = 250 mL;

**P< 0.01 Bonferroni corrected; HT=hormonal replacement therapy, IRSD=Index of Relative Socioeconomic Disadvantage

The most disadvantaged category is indexed by quintile 1

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TABLE 2

Incident fracture rates (n/1000), unadjusted, age-adjusted, and multivariable adjusted HR for MOF in different milk consumption categories with their 95% confidence interval[1]

	Categories of milk consumption[1]			
	No milk	<250 mL/d	250-500 mL/d	>500 mL/d
Number of fractures (n)	24	82	71	29
Person years	1040.0	5001.0	4092.0	1373.4
Rate (n/1000)[2]	23.09	16.40	17.35	21.12
Unadjusted HR	1.40 (0.89, 2.21)[3]	1.00 (reference)	1.05 (0.76, 1.44)	1.28 (0.84, 1.96)
P value	0.15		0.77	0.25
Age adjusted HR	1.54 (0.98, 2.44)	1.0 (reference)	1.00 (0.73, 1.37)	1.23 (0.80, 1.88)
P value	0.06		0.99	0.34
Multivariable adjusted HR[4]	1.56 (0.99, 2.46)	1.0 (reference)	1.02 (0.74, 1.40)	1.15 (0.75, 1.75)
P value	0.06		0.91	0.53

[1] Milk comprises skim, low fat, full fat with a serving size of 1 cup = 250 mL

[2] Fracture rates: number of cases per 1000-person years at risk

[3] 95% CI in parentheses (all such values)

[4] Adjusted for age, oral glucocorticoids, HT, fractures in the past;

HR= hazard ratio, HT= hormonal replacement therapies, MOF=major osteoporotic fracture

TABLE 3

Incident fracture rates (n/1000), unadjusted, age-adjusted and multivariable HR for MOF in different total dairy products consumption categories with their 95% confidence interval[1]

	Categories of total dairy consumption[1]			
	<200 g/d	200-399 g/d	400-799 g/d	≥800 g/d
Fractures	61	66	62	17
Person years	3125.0	4362.1	3492.1	528.1
Rate (per 1000)[2]	14.55	15.10	15.52	17.76
Unadjusted HR	1.30 (0.91, 1.83)[3]	1.00 (reference)	1.18 (0.84, 1.68)	2.10 (1.23, 3.58)
P value	0.15		0.34	0.06
Age adjusted	1.42 (1.00, 2.01)	1.00 (reference)	1.34 (0.94, 1.90)	2.01 (1.18, 3.44)
P value	0.05		0.10	0.01
Multivariable adjusted HR[4]	1.40 (0.98, 1.97)	1.00 (reference)	1.35 (0.95, 1.91)	1.70 (1.00, 2.93)
P value	0.06		0.09	0.05

[1] Total dairy includes milk, cheese, yogurt and ice-cream

[2] Fracture rates: number of cases per 1000-person years at risk

[3] 95% CI in parentheses (all such values)

[4] Adjusted for age, oral glucocorticoids, HT, fractures in the past.

HR= hazard ratio, HT=hormonal replacement therapies, MOF=major osteoporotic fracture

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TABLE 4

Multivariable linear regression analysis of the effects of milk and total dairy consumption categories on serum markers of systemic inflammation and bone turnover with their 95% confidence interval [1]

	hsCRP[2] (mg/L)		CTx[3](ng/L)		P1NP[4](µg/L)	
	Coefficient	95% CI	Coefficient	95% CI	Coefficient	95% CI
Milk consumption categories[5]						
No milk	Reference		Reference		Reference	
< 250 mL/d	-0.29	-0.59, 0.01	-0.15	-0.33, 0.04	-0.10	-0.26, 0.06
250-500 mL/d	**-0.39	-0.70, -0.09	**-0.20	-0.39, -0.02	-0.05	-0.21, 0.11
>500 mL/d	**-0.45	-0.82, -0.07	**-0.25	-0.48, -0.02	-0.13	-0.33, 0.08
Total dairy consumption categories[6]						
< 200 g/d	Reference		Reference		Reference	
200- 399 g/d	0.06	-0.26, 0.15	-0.10	-0.22, 0.03	-0.08	-0.19, 0.02
400- 799 g/d	-0.17	-0.39, 0.04	-0.11	-0.24, 0.01	-0.03	-0.14, 0.10
≥800 g/d	-0.04	-0.44, 0.35	-0.15	-0.39, 0.09	-0.05	-0.27, 0.18

[1] Multivariable linear regression performed on baseline data (cross sectional) of 788 women aged ≥50yr; serum marker of systemic inflammation (hsCRP) and bone turnover (CTx-bone resorption: PINP-bone formation) are log transformed

[2] Model adjusted for BMI, mobility, diabetes, oral glucocorticoids, hypertension

[3] Model adjusted for BMI, age, bisphosphonate, HT

[4] Model adjusted for age, HT, diabetes

[5] Milk comprises skim, low fat, full fat with a serving size of 1 cup = 250 mL

[6] Total dairy includes milk, cheese, yogurt and ice-cream

** P< 0.05.

BMI=body mass index, CTx=C-terminal telopeptide, hsCRP=high sensitivity C-reactive protein, HT=hormonal replacement therapies, P1NP=procollagen type 1 N-terminal propeptide

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FIGURE 1

Kaplan-Meier survival plot for fractures in different milk consumption groups of women. The four curves represent fracture survival probability in different milk consumption groups. The lowest fracture survival probability is shown by the group consuming no milk

FIGURE 2

Kaplan-Meier survival plot for fractures in different total dairy consumption groups of women. The four curves represent fracture survival probability in different total dairy consumption groups. The lowest fracture survival probability is shown by the group consuming ≥800 g/d total dairy

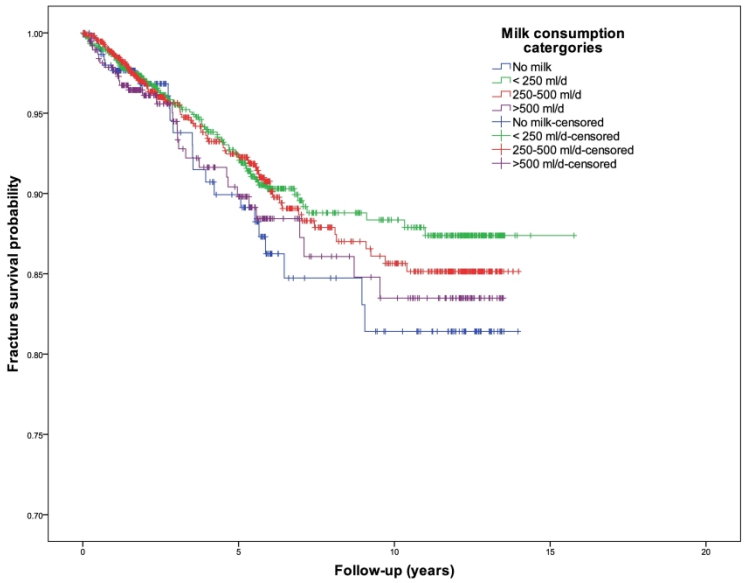

FIGURE 1
Kaplan-Meier survival plot for fractures in different milk consumption groups of women. The four curves represent fracture survival probability in different milk consumption groups. The lowest fracture survival probability is shown by the group consuming no milk

352x211mm (300 x 300 DPI)

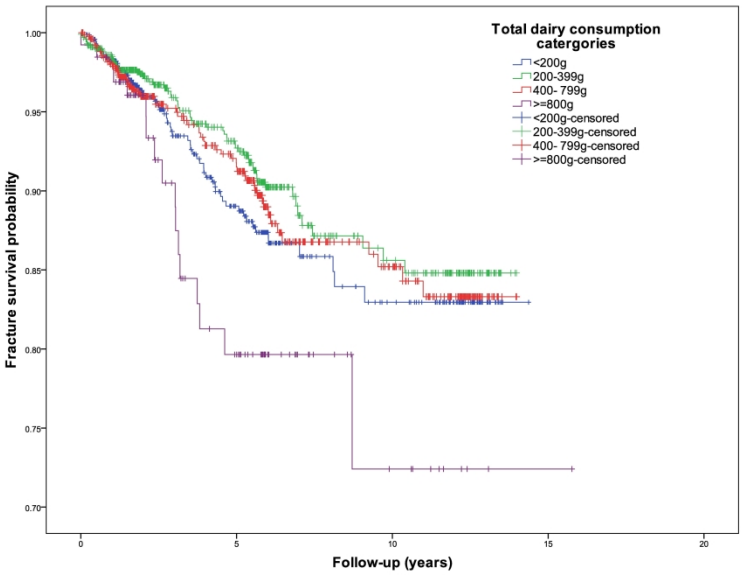

FIGURE 2
Kaplan-Meier survival plot for fractures in different total dairy consumption groups of women. The four curves represent fracture survival probability in different total dairy consumption groups. The lowest fracture survival probability is shown by the group consuming ≥800 g/d total dairy

352x211mm (300 x 300 DPI)

Reporting checklist for cohort study.

Based on the STROBE cohort guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the STROBE cohortreporting guidelines, and cite them as:

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		Reporting Item	Page Number
Title and abstract			
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	1
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	#3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	#4	Present key elements of study design early in the paper	5
Setting	#5	Describe the setting, locations, and relevant dates, including periods of	5

		recruitment, exposure, follow-up, and data collection	
Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up.	5
Eligibility criteria	#6b	For matched studies, give matching criteria and number of exposed and unexposed	N/A
Variables	#7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6,7,8,9
Data sources / measurement	#8	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable.	6,7,8,9
Bias	#9	Describe any efforts to address potential sources of bias	16
Study size	#10	Explain how the study size was arrived at	17
Quantitative variables	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	10,11
Statistical methods	#12a	Describe all statistical methods, including those used to control for confounding	10,11
Statistical methods	#12b	Describe any methods used to examine subgroups and interactions	N/A
Statistical methods	#12c	Explain how missing data were addressed	
Statistical methods	#12d	If applicable, explain how loss to follow-up was addressed	17
Statistical methods	#12e	Describe any sensitivity analyses	N/A
Results			
Participants	#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable.	N/A

Participants	#13b	Give reasons for non-participation at each stage	
Participants	#13c	Consider use of a flow diagram	
Descriptive data	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable.	11/24
Descriptive data	#14b	Indicate number of participants with missing data for each variable of interest	
Descriptive data	#14c	Summarise follow-up time (eg, average and total amount)	12/16
Outcome data	#15	Report numbers of outcome events or summary measures over time. Give information separately for exposed and unexposed groups if applicable.	
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	11/12
Main results	#16b	Report category boundaries when continuous variables were categorized	
Main results	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	11
Discussion			
Key results	#18	Summarise key results with reference to study objectives	13
Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	17
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	18
Generalisability	#21	Discuss the generalisability (external validity) of the study results	18
Other Information			

Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	18

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1

Association Between Dairy Intake And Fracture In An Australian Based Cohort Of Women

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Abstract

Objective: Given the inconsistent evidence on dairy consumption and risk of fracture, we assessed the association between milk/total dairy consumption and major osteoporotic fracture (MOF) in women from the Geelong Osteoporosis Study (GOS).

Methods: Women aged ≥50yr (n= 833) were followed from baseline (1993-1997) to date of first fracture, death, or 31 December 2017, whichever occurred first. Milk/total dairy (including milk, cheese, yogurt and ice-cream) consumption was assessed by self-report. Major Osteoporotic fractures (MOFs) (hip, forearm, clinical spine and proximal humerus) were confirmed radiologically. Multivariable adjusted Cox proportional hazard models were used to determine associations between milk/total dairy consumption and MOFs. Cross-sectional associations between milk/total dairy consumption and serum high sensitivity C-reactive protein (hsCRP), C-terminal telopeptide (CTx) and procollagen type 1 N-terminal propeptide (P1NP) at baseline were investigated using multivariable linear regression.

Results: During follow-up (11,507 person-years), 206 women had a MOF. Consuming >500 mL/d of milk was not significantly associated with increased HR for MOF. Non-milk consumers had higher hazard ratio (HR) for MOF (1.56; 95% CI 0.99, 2.46) compared to consuming <250mL/d of milk. Consuming ≥800 g/d total dairy increased the HR for MOF (1.70; 95% CI 1.00, 2.93) compared to consuming 200-399g/d. Milk consumption was inversely associated with serum hsCRP and CTx but total dairy consumption was not associated with these serum markers.

Conclusion: Evidence was not found to show that higher milk consumption increased the risk for MOF in older women. However, zero milk consumption and higher total dairy consumption increased the risk for MOF.

Key words: Fractures, Milk, Osteoporosis, Dairy, Inflammation

Strengths and limitations of the study

⇒ Although this study contained a modest sample size, it replicated the findings of previous studies.

⇒ Random sample selection from the general population is a strength of the study

⇒ The prospective study design strengthens the outcomes of the study despite methodological inconsistencies in capturing dietary data.

⇒ As data for total dairy consumption were assessed at baseline only, we cannot account for dietary changes during follow-up and this limits the interpretation of the longitudinal analysis of the association between total dairy consumption and the risk for major osteoporotic fracture

⇒ The conclusions of this study cannot be generalised to a broader population as this study was focused on a cohort of women.

Introduction

Osteoporosis is a chronic multifactorial disease, that is defined as low bone mass and impaired bone micro-architecture (1, 2). The presence of osteoporosis substantially increases the risk of sustaining a fracture, especially at the hip, spine, forearm and proximal humerus, which are known as the major osteoporotic fracture (MOF) sites. However, the largest absolute number of fractures occurs in people with a moderate deficit in bone density (osteopenia) (3, 4). Falls risk, which can be affected by factors such as medication use, mobility level and environmental hazards is also an important consideration, as most fractures are preceded by a fall (5-8). Of the factors (e.g. genetics (9, 10), age (11, 12), lifestyle habits (2), sex (13)) influencing fractures, nutrition plays a substantial role in the aetiology of osteoporosis (14, 15). Adequate calcium and protein intakes are necessary in order to maintain skeletal integrity and strength (16, 17). Milk/dairy products are key components in the western diet and contain a myriad of nutritional components (calcium, vitamins and proteins) and a majority of an individual's dietary calcium needs are fulfilled by intake of dairy products (18, 19). Additionally, milk/dairy products have been widely recommended to osteoporosis patients by clinicians and healthcare professionals considering the beneficial effects associated with dairy consumption (20, 21).

However, data regarding milk consumption as a strategy for fracture prevention has shown inconclusive results. Findings from large Swedish cohorts reported that women who consumed three or more glasses of milk per day had higher risk for any fractures while fermented dairy consumption was inversely associated with fractures (22). However, Feskanich *et al.* have shown in two large US cohorts that each serving of milk per day was associated with an 8% reduction of risk for hip fracture, whereas total dairy intake was associated with a 6% reduction of risk for hip fractures in men and women combined (23). Holvik *et al*. found no association between increased milk intake and risk for hip fractures in Norwegian women and men (24). The most recently published meta-analysis (2018), which included 18 observational studies,

showed that higher milk intakes were not associated with fractures in both sexes combined (25). However, it is worth noting that there was a large amount of heterogeneity between studies in terms of reporting milk/dairy intake, number of fractures, use of different confounders for adjustment and fracture ascertainment methods.

Due to the burden of osteoporosis in women (3) and the inconclusive nature of the results in the field, we aimed to assess the association between milk/total diry (e.g. milk, cheese, yogurt and ice-cream) consumption and risk for major osteoporotic fracture (MOF) in a sample of Australian women. We hypothesised that increased milk and total dairy consumption may be associated with increased risk for MOF. We also investigated potential mechanisms by which increased milk/total dairy may mediate the risk for MOF. For this purpose, the cross sectional association between milk/total dairy consumtion, and serum high sensitivity C-reactive protein (hsCRP), C-terminal telopeptide (CTx) and procollagen type 1 N-terminal propeptide (P1NP) were examined at baseline.

Methods

Patient and Public involvement

Patients were not involved in the planning and design of the study.

Study Population

This study used data from the Geelong Osteoporosis Study (GOS), a large population-based cohort study based in south-eastern Australia. Inclusion criteria were: living in the Barwon Statistical Division (BSD) for > 6 months and able to provide written informed consent. Women in the BSD were selected at random from the electoral roll during the years 1993-1997 to participate in the study (26). An age-stratified sample of 1,494 women was enrolled in the study with a participation of 77.1%. Subsequent assessments for these women commenced in

1995, 1998, 2000, 2002, and 2004, referred to as 2-year, 4-year, 6-year, 8-year, and 10-year follow-up phases. The cohort profile is explained elsewhere (26). For the purposes of the analysis women only ≥50yr at baseline were considered. Of the 836 women aged ≥ 50 yr, 833 women were included in the analysis after excluding records with missing information on milk intake (Figure1). Study participants provided written informed consent. The study was approved by the Human Research Ethics Committee at Barwon Health.

Outcome Measures

Post-baseline incident fractures were identified using a method that have been validated for fracture ascertainment in the region. Radiological reports (X-ray) of fractures from all radiological centres in the region were scrutinised to identify and confirm fractures. (27, 28). Trained research personnel examined each record individually and determined the most appropriate international code of diseases version 9 (ICD-9) codes for fracture site, as well as level of trauma (29). MOFs were defined as fractures at the hip, forearm, clinical spine and proximal humerus, according to the fracture risk assessment tool (FRAX) developed by the University of Sheffield for clinical use (30). Pathological and high trauma fractures were excluded. Information on death was collected from the National Deaths Index (Australian Institute for Health and Welfare).

Dairy consumption and diet

Information on dairy was available at baseline, 6 year and 10-year follow-up. At baseline and 6-year follow-up, dietary information was documented by a self-reported questionnaire that contained questions on 35 foods and beverages on average. Participants were asked questions about the usual (habitual) type of milk consumed (whole, reduced fat, calcium fortified, soy, goat's milk, butter milk, and evaporated) and the quantity consumed each day. In the questionnaire, it was stated that one cup of milk is considered equivalent to 250 mL. Therefore,

participants chose the type and quantity of milk consumed from any pre-determined milk categories and only cow's milk was considered (none, < 125 mL (< ½ cup), 125 -249 mL (½-<1 cup), 250-499 mL (1-<2 cups), 500- 999 mL (2-<4 cups), ≥ 1000 mL (≥ 4 cups) per day). The lowest response categories <125 mL/d, 124-249 mL/d were collapsed into one category indicating "< 250 mL/d" and the highest response categories, 500- 999 mL/d, ≥ 1000 mL were combined into one category indicating "> 500 mL/d"; this was due to low proportions responding to the lower and higher categories and for the compatibility with the 10-year follow-up dietary data. Information on other dairy products such as cheese, yogurt and ice-cream consumption were also documented using this self-reported questionnaire. Participants were specifically asked about different types of cheese they consumed on a weekly basis including hard cheese (servings/week; 1 serving = 16 g); soft cheese (servings/week; 1 serving= 20 g); and fruche (servings/week; 1 serving = 100 g). Fruche is a form of soft cheese (fromage frais) and thus was categorised as cheese. Total cheese consumption was converted to grams consumed per day (g/d). Yogurt (servings/week; 1 serving= 200 g) and ice-cream consumption (servings/week; 1 serving = 27 g) were reported as servings per week and this was converted to grams consumed per day. For the purpose of this study, daily total dairy consumption was calculated at baseline by combining values for cow's milk, all forms of cheese, yogurt and ice-cream consumed and was expressed in grams consumed per day. Further, total dairy consumption was categorised as < 200 g/d, 200-399 g/d, 400-700 g/d, ≥800 g/d.

At 10-year follow-up, information on milk/dairy consumption was collected using a validated food frequency questionnaire. The Cancer Council Victoria Dietary Questionnaire captures information on 74 foods and six alcoholic beverages over the previous 12 months and is validated for assessing habitual dietary intake in Australian women (31). Participants were queried on their usual type (none, full cream, reduced fat, skim, and soymilk) and quantity of milk consumed on a daily basis. Participants were advised that 1 cup of milk is equivalent to

250 mL of milk. Furthermore, participants indicated their daily milk intake by selecting from pre-determined categories of milk intakes and only cow's milk was considered (none, < 250 mL (<1cup), 250- 499 mL (1-<2 cups), 500- 750 mL (2-3 cups) and > 750 mL (> 3 cups) per day). The highest response categories, 500-750 mL/d, > 750 mL/d were combined as to one category indicating "> 500 mL/d"; this was due to low proportions responding to the higher categories. This questionnaire also captured information on cheese, yogurt and ice-cream intake of participants.

A separate calcium-specific dietary questionnaire was used to capture information on dietary calcium intake. This questionnaire included information on a range of common calcium-dense food sources, which allowed calculation of dietary calcium intakes in mg per day (mg/d) and validated against 4-day weighed food intakes (19). Dietary calcium intake was categorised into two strata (< 1000 mg/d, ≥ 1000 mg/d).

Other information and potential confounders

All measurements were assessed at the baseline visit. Weight and height were recorded to the nearest 0.1 kg and 0.1 cm respectively and body mass index (BMI) calculated as weight/height^2 (kg/m^2). Dual-energy x-ray absorptiometry (DXA; Lunar DPX-L; Lunar, Madison, WI) was performed to evaluate bone mineral density (BMD; g/cm^2) at the femoral neck, and whole-body fat (kg), and 'lean' mass (kg) which represents the water and protein content in muscle, skin, connective tissue and lean component in adipose tissue.

Self-report questionnaires were used to obtain information on mobility, physical activity levels, smoking status, medications, prior falls, and fractures. Participants were asked to select their mobility level from pre-determined 7-point scale (very active, active, sedentary, limited, inactive, chair or bed ridden, bedfast - examples were given in the questionnaire to assist the participant to choose the most suitable option). These categories were further condensed to two

groups, highly active and less active, for the purpose of this analysis. Physical activity level was also assessed from questions regarding work/home and recreational/sports, on a 3 point-scale which provided options for participants to select from moderate, hard and very hard. Participants were also asked to enter the time spent on each activity level on a weekly basis.

Information on current smoking status was categorised as smoking or non-smoking. Use of medications that positively or negatively influence bone included bisphosphonates, anabolic therapies, hormonal replacement therapies (HT), and oral glucocorticoids. Participants were asked to list the use of supplements and this information was used to assess the calcium and vitamin D supplementation usage. Use of supplementary calcium and vitamin D were documented at baseline, 6 yr and 10 yr follow-up.

The definition of falls (when you suddenly find yourself on the ground, without intending to get there, after you were in either a lying, sitting or standing position) was explicit in the questionnaire and asked participants whether or not they experienced a similar scenario over the past 12 months. Information regarding previous fractures and cancer diagnoses was also captured by self-reported questionnaires. An automated device (Takeda Medical UA-751) was used to measure blood pressure in a sitting position. Women were considered hypertensive if they had a systolic blood pressure over 140 mmHg and/or a diastolic pressure above 90 mmHg and/or use of antihypertensive medication in the presence of self-reported hypertension. Women were identified as having diabetes if they had a fasting plasma glucose ≥ 7.0 mmol/L (126 mg/dL), self-reported diabetes and/or use of antihyperglycaemic agents.

Information pertinent to educational qualifications were gathered on a 7-point scale: never attended school, primary school, some secondary school, completed secondary school, post-secondary qualifications, university or other tertiary qualifications, and can't remember. These categories were compressed to education received for less than 12 years or more than 12 years for the purpose of this analysis. Information on marital status was dichotomised as living alone

or living with a partner. The socio-economic status of the cohort participants was measured by the Index of Relative Socioeconomic Disadvantage (IRSD), an area-based index that measures relative disadvantage of socio-economic status. This tool imputes a span of information on economic and social conditions of people and household within an area and is represented in quintiles. The most disadvantaged category is indexed by quintile 1 (32).

Biomarkers

At baseline, venous blood was collected after an overnight fast and stored at -80 ^{0}C until batch analysis. Markers of bone turnover, serum C-terminal telopeptide (CTx), a marker of bone resorption, and serum procollagen type 1 N-terminal propeptide (P1NP), a marker of bone formation, were analysed from the blood samples. In addition, high sensitivity serum C-reactive protein (hsCRP), a marker of systemic inflammation, was determined from the blood samples. Serum hsCRP was measured by the Roche immunoturbidometric 'CRP" and 'C-reactive protein (latex) high sensitivity methods. Details of these analytical methods have been described elsewhere (33, 34).

Statistical analysis

Characteristics of participants were described by mean (± SD) or median (IQR) or relative frequencies (%) stratified by milk consumption categories (no milk, <250 mL/d, 250-500 mL/d, > 500 mL/d). Participant characteristics across categories of milk consumption were compared using one-way ANOVA or Kruskal-Wallis-H test for continuous data and Chi-square test (or Fisher's exact test) for categorical data. The null hypothesis was rejected at an α level of 0.05 and a post hoc multiple comparison was performed using Bonferroni corrections. Additionally, participant characteristics were described based on total dairy categories (<200 g/d, 200-399 g/d, 400-799g/d, ≥ 800 g/d) (Supplementary Table 1).

Cohort participants were followed from their baseline appointment to date of first fracture, death, or 31 December 2017. Cox proportional hazard regression was used to estimate age adjusted hazard ratio (HR) and their 95% confidence intervals for categories of milk consumption (no milk, < 250 mL/d, 250-500 mL/d, > 500 mL/d).

Covariates (BMD, BMI, smoking, alcohol consumption, pre-baseline fractures incidents, diabetes, IRSD, education, mobility, medications that influence bone metabolism, calcium and vitamin D supplements) were assessed in bivariate Cox regression analysis to determine their impacts on the association between milk/total dairy consumption and fractures. The covariates that impacted the hazard ratio when added or removed (considering the statistical significance and change of HR in the exposure of interest) from the model were included in the final Cox regression model. In addition, when deciding on the confounders, the potential of the covariate to be associated with both the exposure and outcome was also considered. The final model consisted, age, oral glucocorticoid use, HT use and pre-baseline factures as confounders. Information on milk consumption, oral glucocorticoid use, and HT use were time updated at the 6 and 10-year follow-up. Age was time updated in all follow-up waves. Information on pre-baseline fractures were not time updated and kept constant for the analysis. We also performed a multivariable adjusted sensitivity analysis using baseline milk values only.

In addition, a Cox proportional hazard regression was also used to estimate the age adjusted HR and their 95% confidence intervals for total dairy consumption categories (< 200 g/d, 200-399 g/d, 400-799 g/d, ≥ 800 g/d). Selection of potential confounders was performed according to the aforementioned method. The final model consisted age, oral glucocorticoid use, HT use, and prior fractures as confounders. For this analysis total dairy consumption was not time updated due to the inconsistent dietary tools used in capturing information on dairy products during the follow-up waves; therefore, baseline only values for total dairy consumption was

used in the analysis. Oral glucocorticoid use and HT use were time updated at the 6 and 10-year follow-up; age was time updated at all follow-up waves; pre-baseline fractures were not time updated and kept constant for the analysis.

The proportional hazard assumptions were confirmed graphically by log(-log(survival)) plots for both daily milk and total dairy consumption. Time to first fracture (survival) curves were illustrated using Kaplan-Meier estimator of the survival function using product limit estimator.

The cross-sectional associations between milk/total dairy consumption and serum markers of inflammation (hsCRP) and bone turnover (CTx, P1NP) were assessed using multivariable linear regression models at baseline with potential confounders. Women who had missing information (n=45) on inflammatory and bone turnover markers were excluded from the analysis. Serum markers of inflammation and bone turnover were log transformed due to the skewed nature of data. For all analyses, STATA 15 and SPSS 25 was used.

Results

Descriptive characteristics of the cohort stratified by milk consumption categories are presented in Table 1. Of 833 women, 8.4% (*n*=70) did not consume milk and 47.2% (*n*=393), 34.3% (*n*=286) and 10.0% (*n*=84) consumed < 250 mL/d, 250-500 mL/d and > 500 mL/d of milk, respectively. There was no difference observed in women's median age among the four-milk consumption categories. Women who consumed >500 mL/d of milk reported the highest cheese intake. The group that consumed < 250 mL/d of milk had the highest proportion of women reporting < 1000 mg/d of dietary calcium intake. On the other hand, the group that consumed > 500 mL/d of milk had the highest proportion of women reporting ≥1000 mg/d of dietary calcium intake (Table 1). The proportion of women consuming supplementary calcium and vitamin D was high among the non-milk consumers. There were no differences detected for other parameters across the four-milk consuming groups.

During 11,507-person years of follow-up, 206 women sustained a MOF (spine=96; humerus=14; wrist=51; hip=45) and 503 women died. Women who consumed no milk reported the highest fracture rate (Table 2) and the crude fracture survival probability curve also showed that women who consumed no milk had the lowest survival probability for fractures (Figure 2). Concordantly, women who reported no milk consumption showed marginally significant higher age adjusted (1.54, 95% CI 0.98, 2.44, P= 0.06) and multivariable adjusted (1.56, 95% CI 0.99, 2.46, *P*=0.06) HR for MOF compared to women who consumed < 250 mL/d of milk. The unadjusted (1.28, 95% CI 0.84,1.96, *P*=0.25), age adjusted (1.23, 95% CI 0.80,1.88, *P*=0.34), and multivariable adjusted (1.15, 95% CI 0.75,1.75, *p*=0.53) HR for MOF were not significantly higher in women who consumed > 500 mL/d of milk compared to women who consumed < 250 mL/d of milk. The multivariable adjusted sensitivity analysis, which was performed using baseline milk values only resulted non-significant higher HR ratio for non-milk consumers (HR:1.53; CI: 0.96-2.44; *p*=0.07) and >500 mL/d of milk consumers (HR:1.13; CI:0.74-1.72; *p*=0.58) compared to consuming < 250 mL/d milk of milk.

When total dairy consumption was considered, women who consumed more than ≥ 800 g/d demonstrated the highest fracture rate (Table 3). This was also confirmed by the crude fracture survival probability curve, which indicated the lowest survival probability for fractures in women who consumed ≥ 800 g/d total dairy (Figure 3). Consistently, women who consumed ≥ 800g/d total dairy showed higher age adjusted (2.01, 95%CI 1.88, 3.44, *P*=0.01) and multivariable adjusted (1.70, 95% CI 1.00, 2.93, *P*=0.05) HR for MOF compared to women who consumed 200-399 g/d of total dairy (Table 3).

An inverse association was observed between milk consumption and serum markers of inflammation (hsCRP) and serum markers of bone resorption (CTx); women who consumed > 500 mL/d of milk had the lowest concentrations of serum hsCRP (-0.45; 95%CI: -0.82, -0.07; *P*= 0.02) and serum CTx (-0.25; 95% CI: -0.48, -0.02; *P*= 0.03) (Table 4). No association was

found between milk consumption and serum marker of bone formation (P1NP). Moreover, there was no association found between total dairy consumption categories and serum hsCRP, CTx and P1NP (Table 4).

Discussion

In our study of older Australian women, we detected no significant association between higher milk consumption (> 500 mL/d) and increased risk for MOF. However, we found that zero milk consumption was associated with increased risk for MOF. In addition, our study results demonstrated that consuming higher amount of total dairy (≥ 800 g/d) was associated with an increased risk for MOF.

Acquiring the daily recommend calcium through diet/supplements is considered the easiest and safest lifestyle modification that could be achieved as a part of prevention and management of osteoporosis (35). Milk/dairy products are considered the ideal source of calcium that consumed in recommended quantities, may approximately satisfy the daily calcium requirements (36, 37). In general, 1200 mg/d of calcium is recommended for women aged > 50yr (38) and potentially four serves of milk (1 serve = 250 mL = 300 mg of calcium) can cover this need. Our study results revealed that consuming no milk was associated with increased risk of MOF.

However, some components in milk such as D-galactose, a milk sugar (39) and A1-beta-casein, a mutated form of milk casein (40) are believed to possibly mediate the unfavourable consequences associated with milk consumption. D-galactose has proven to be involved in the ageing process in mice, which encompassed series of events such as oxidative stress and chronic inflammation (41). Besides, existing epidemiological data show that some negative health consequences (Ischemic heart disease and Type 1 diabetes) associated with milk

consumption may be due to the A1 beta-casein fraction in milk (42-46). However, robust evidence from clinical trials are lacking to confirm causality.

The Swedish cohort study speculated that increased milk intake may be deleterious to bone due to the D-galactose content in milk, and showed that women who consumed more than 3 glasses of milk compared to 1 glass of milk per day had higher risk for any fractures and mortality (22). Additionally, a positive correlation between milk consumption and both oxidative stress marker in urine (8-iso-PGF2α) and inflammatory marker in serum (IL-6) were detected in the Swedish cohort (22). Hence, those findings offered support to the hypothesis that increased milk intakes are deleterious to bone and this may be mediated through D-galactose in milk (22). However, many other studies (24, 25, 47) including our study did not find any evidence to show that increased milk consumption is associated with fractures. We also assessed the association between total dairy consumption (milk, cheese, yogurt and ice-cream) and MOF. Here, we found that women who consumed ≥ 800 g/d of total dairy showed higher risk for MOF compared to women consuming moderate levels.

This study also attempted to assess the underpinning mechanisms by, which higher milk/total dairy products may instigate fractures. We hypothesised that increased milk/total dairy intakes may augmented systemic inflammation; thereby negatively influence bone metabolism, and increase bone fragility and risk for fractures (34, 48). Serum hsCRP is deemed a sensitive marker of systematic inflammation and higher concentration of serum hsCRP has been detected in inflammatory diseases and also associated with fractures(49, 50). Therefore, the cross-sectional association between milk/total dairy categories and serum marker of inflammation (hsCRP) was tested. The lowest serum hsCRP concentration was detected in women who consumed > 500 mL/d of milk. Our findings did not support our hypothesis and they were corroborated by other literature that showed decreased CRP levels with increased milk/dairy intake (51). Also, we did not find any association between total dairy consumption and serum

hsCRP. We also, assessed whether there is an association between milk/total dairy consumption categories and markers of bone turnover. There were no clear patterns of associations found between milk consumption and serum marker of bone formation (P1NP). But women consuming >500 mL/d of milk had the lowest concentrations of serum marker of bone resorption (CTx). Moreover, there was no association detected between total dairy consumption and serum CTx and P1NP.

Our study has several strengths. One strength of the study is, GOS comprise a randomly selected group of participants, which has shown to be similar to the broader Australian population (in terms of income, SES, etc.). However, our findings are not generalisable to men, nor other countries with different distributions of ethnicities, diet patterns and other factors. However, our study results are likely generalisable to the broader Australian population of women. We were able to perform a longitudinal analysis that incorporated a long follow-up time with a median of 14.26 years as GOS is a cohort study. Additionally, data on the main exposure variable and confounders were updated several times during the period of follow-up, which enhanced the robustness of our analyses. Cognisant that osteoporosis is a multifactorial disease, we included many possible potential confounders (age, oral glucocorticoids, HT, and past fractures) in the analysis. Also, we used an objective method of ascertaining/confirming incident fractures from radiological reports rather than relying on self-reported information. However, this was a regionally validated fracture ascertainment method, which may not account for fractures in participants who left the region (Figure 1).

However, our study did have some limitations. We were unable to describe the association between milk/total dairy intake and fracture risk, as U/J shaped graphs showing higher risks in the zero and high consumption groups and the lowest risk in the low-intermediate consumption groups because of the low number of fractures, which may lead to lower precision in the estimates. Also, total dairy consumption in the Cox regression analysis, was not time update

as dietary information was not collected consistently across all follow-up visits and we thus performed an analysis using the baseline data only. This might have led to unaccounted changes in exposure status that may have occurred during the period of follow-up. The study sample size was modest. A post-hoc power calculation showed that based on annual fracture rate of 14.10 per 1,000 in the reference group (<250 mL/d milk consumption) the minimum detectable effect size (i.e. RR) ranged from 1.5 to 1.9, which was bigger than observed risk ratios from unadjusted and adjusted Cox models. In addition, some participants were lost to follow-up during the study due to leaving the region (n=29), which prevented time-dependent updates on their information. As with all observational/follow-up studies, attrition is unavoidable. In the interim, there may have been other unrecognised confounding in our study. We also did not record changes in the type of milk consumed over the duration of follow-up and could not differentiate between exposure to conventional dairy products or A2 milk products; thus, we were not able to investigate particular milk proteins as mediating potential negative effects associated with milk consumption.

Conclusion

Taken together, our study results suggest that higher milk consumption is not associated with increased risk for MOF; however, zero milk consumption appears to be associated with an increased risk for MOF. Also, higher consumption of total dairy (milk, yogurt, cheese and ice-cream) may increase the risk for MOF, indicating a negative influence on bone health. Further studies are warranted to identify optimal levels of milk and total dairy consumption ranges and the potential mechanisms by which total dairy consumption may influence the risk for fracture.

Contribution: HA, KLH-K, MM, FNJ, JAP contributed to the interpretation of data, and critical appraisal of the manuscript and HA constructed the manuscript.

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Ethical approval: The study was approved by the Human Research Ethics Committee at Barwon Health.

Data sharing statement: Data for this study will be available upon request. Request can be sent to gos@barwonhealth.org.au.

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TABLE 1

Baseline characteristics of participants stratified by milk consumption categories[1]

	No milk	< 250 ml/d	250-500 ml/d	> 500 ml/d
Number of women	70	393	286	84
Age at entry, yr	68.2 (58.2-77.6)	69.1 (59.2-80.3)	71.4 (60.5-80.4)	71.7 (64.2-80.4)
Body mass index, kg/m^2	25.1 (22.1-28.6)	26.8 (24.1-30.3)	25.9 (23.5-29.9)	25.3 (23.2-28.9)
Yogurt, g/d	0.0 (0.0-57.1)	0.0 (0.0-57.1)	3.6 (0.0-57.1)	0.0 (0.0-85.7)
Cheese, g/d	9.1 (3.4-22.9)	9.1 (4.6-16.0)	11.0 (4.6-22.9)	**13.7 (6.9-25.1)
Ice-cream, g/d	0.0 (0.0-11.6)	0.0 (0.0-7.7)	0.0 (0.0-7.7)	0.0 (0.0-11.6)
Bone mineral density, g/cm^2	0.792 ± 0.163	0.830 ± 0.156	0.832 ± 0.146	0.808 ± 0.161
Whole-body fat, kg	24.1 (18.9-32.2)	27.6 (20.7-34.0)	25.7 (20.5-32.6)	24.6 (19.5-29.0)
Lean mass, kg	36.3 ± 4.8	37.3 ± 4.7	37.3 ± 4.6	36.9 ± 4.1
Dietary calcium, *n* (%)				
<1000 mg/d	65 (93)	**386 (98)	246 (86)	1(1)
≥1000 mg/d	5 (7)	4 (1)	39 (13)	**82 (98)
Falls in the past, *n* (%)	14 (20)	73 (19)	63 (20)	21 (25)
Pre-baseline fractures, *n* (%)	24 (34)	146 (37)	93 (33)	39 (46)
Incident cancer, *n* (%)	7 (10)	58 (15)	37 (13)	14 (17)

Diabetes, *n* (%)	6 (9)	30 (8)	22 (8)	11 (13)
Hypertension, *n* (%)	47 (67)	242 (62)	172 (60)	51 (61)
Smoking, *n* (%)				
Smokers	64 (91)	348 (89)	267 (93)	78 (93)
Non-smokers	6 (9)	45 (11)	19 (7)	6 (7)
Mobility, *n* (%)				
Highly active	38 (54)	192 (49)	142 (50)	43 (51)
Less active	32 (46)	201 (51)	144 (50)	41 (49)
Supplemental calcium, *n* (%)	**16 (22)	49 (12)	34 (12)	17 (20)
Supplemental vitamin D, *n* (%)	**15 (21)	45 (11)	25 (9)	15 (18)
Bisphosphonates, *n* (%)	1 (1)	1 (0)	6 (2)	0 (0)
Anabolic therapies, *n* (%)	0 (0)	0 (0)	0 (0)	0 (0)
HT, *n* (%)	10 (14)	70 (18)	38 (13)	8 (10)
Oral glucocorticoids, *n* (%)	2 (3)	9 (3)	5 (2)	6 (7)
IRSD *n* (%) / quintile				
1	11(15)	81(21)	48 (17)	15 (18)
2	18 (26)	81(21)	63 (22)	19 (24)
3	16 (23)	97 (25)	58 (20)	20 (24)

4	12 (17)	58 (14)	55 (19)	14 (17)
5	13 (19)	76 (19)	62 (22)	16 (19)
Education, *n* (%)				
<12 years	63 (90)	342 (87)	248 (87)	69 (82)
≥12 years	7 (10)	46 (11)	37 (13)	14 (17)
Marital status, *n* (%)				
Living with partner	28 (40)	162 (41)	137 (48)	43 (51)
Living alone	42(60)	231(59)	149 (52)	41 (49)

[1] Data reported as mean± SD, median (IQR) or n (%); Milk comprises skim, low fat, full fat with a serving size of 1 cup = 250 mL;
**P< 0.01 Bonferroni corrected; HT=hormonal replacement therapy, IRSD=Index of Relative Socioeconomic Disadvantage
The most disadvantaged category is indexed by quintile 1

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TABLE 2

Incident fracture rates (n/1000), unadjusted, age-adjusted, and multivariable adjusted HR for MOF in different milk consumption categories with their 95% confidence interval[1]

	Categories of milk consumption[1]			
	No milk	<250 mL/d	250-500 mL/d	>500 mL/d
Number of fractures (n)	24	82	71	29
Person years	1040.0	5001.0	4092.0	1373.4
Rate (n/1000)[2]	23.09	16.40	17.35	21.12
Unadjusted HR	1.40 (0.89, 2.21)[3]	1.00 (reference)	1.05 (0.76, 1.44)	1.28 (0.84, 1.96)
Age adjusted HR	**1.54 (0.98, 2.44)	1.0 (reference)	1.00 (0.73, 1.37)	1.23 (0.80, 1.88)
Multivariable adjusted HR[4]	**1.56 (0.99, 2.46)	1.0 (reference)	1.02 (0.74, 1.40)	1.15 (0.75, 1.75)

[1] Milk comprises skim, low fat, full fat with a serving size of 1 cup = 250 mL (time updated at 6 year and 10year follow-up waves)

[2] Fracture rates: number of cases per 1000-person years at risk

[3] 95% CI in parentheses (all such values)

[4] Adjusted for oral glucocorticoids, HT, (time updated at 6, 10-year follow-up waves), age (time updated at all follow-up waves) pre-baseline fractures (baseline values)

HR= hazard ratio, HT= hormonal replacement therapies, MOF=major osteoporotic fracture (fractures in hip, forearm, clinical spine and proximal humerus)

**p <0.05

TABLE 3

Incident fracture rates (n/1000), unadjusted, age-adjusted and multivariable HR for MOF in different total dairy products consumption categories with their 95% confidence interval[1]

	Categories of total dairy consumption[1]			
	<200 g/d	200-399 g/d	400-799 g/d	≥800 g/d
Fractures	61	66	62	17
Person years	3125.0	4362.1	3492.1	528.1
Rate (per 1000)[2]	19.52	15.13	17.75	32.19
Unadjusted HR	1.30 (0.91, 1.83)[3]	1.00 (reference)	1.18 (0.84, 1.68)	2.10 (1.23, 3.58)
Age adjusted	1.42 (1.00, 2.01)	1.00 (reference)	1.34 (0.94, 1.90)	**2.01 (1.18, 3.44)
Multivariable adjusted HR[4]	1.40 (0.98, 1.97)	1.00 (reference)	1.35 (0.95, 1.91)	**1.70 (1.00, 2.93)

[1] Total dairy includes milk, cheese, yogurt and ice-cream

[2] Fracture rates: number of cases per 1000-person years at risk

[3] 95% CI in parentheses (all such values)

[4] Adjusted for oral glucocorticoids, HT, (time updated at 6, 10year follow-up waves), age (time updated at all follow-up waves) pre-baseline fractures (baseline values)

HR= hazard ratio, HT=hormonal replacement therapies, MOF=major osteoporotic fracture (fractures in hip, forearm, clinical spine and proximal humerus)

**p <0.05

TABLE 4

Association between milk/total dairy consumption categories, and serum markers of systemic inflammation and bone turnover with their 95% confidence interval [1]

	hsCRP[2] (mg/L)		CTx[3](ng/L)		P1NP[4](µg/L)	
	Coefficient	95% CI	Coefficient	95% CI	Coefficient	95% CI
Milk consumption categories[5]						
No milk	Reference		Reference		Reference	
< 250 mL/d	-0.29	-0.59, 0.01	-0.15	-0.33, 0.04	-0.10	-0.26, 0.06
250-500 mL/d	**-0.39	-0.70, -0.09	**-0.20	-0.39, -0.02	-0.05	-0.21, 0.11
>500 mL/d	**-0.45	-0.82, -0.07	**-0.25	-0.48, -0.02	-0.13	-0.33, 0.08
Total dairy consumption categories[6]						
< 200 g/d	Reference		Reference		Reference	
200- 399 g/d	0.06	-0.26, 0.15	-0.10	-0.22, 0.03	-0.08	-0.19, 0.02
400- 799 g/d	-0.17	-0.39, 0.04	-0.11	-0.24, 0.01	-0.03	-0.14, 0.10
≥800 g/d	-0.04	-0.44, 0.35	-0.15	-0.39, 0.09	-0.05	-0.27, 0.18

[1] Multivariable linear regression performed on baseline data (cross sectional) of 788 women aged ≥50yr; serum marker of systemic inflammation (hsCRP) and bone turnover (CTx-bone resorption: P1NP-bone formation) are log transformed

[2] Model adjusted for BMI, mobility, diabetes, oral glucocorticoids, hypertension

[3] Model adjusted for BMI, age, bisphosphonate, HT

[4] Model adjusted for age, HT, diabetes

[5] Milk comprises skim, low fat, full fat with a serving size of 1 cup = 250 mL

[6] Total dairy includes milk, cheese, yogurt and ice-cream

** P< 0.05.

BMI=body mass index, CTx=C-terminal telopeptide, hsCRP=high sensitivity C-reactive protein, HT=hormonal replacement therapies, P1NP=procollagen type 1 N-terminal propeptide

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FIGURE 1

Participant flow chart. The figure represents the number of women at baseline, 6 and 10-year follow-up waves, and women left the region.

FIGURE 2

Kaplan-Meier survival plot for fractures in different milk consumption groups of women. The four curves represent fracture survival probability in different milk consumption groups (crude data). The lowest fracture survival probability is shown by the group consuming no milk.

FIGURE 3

Kaplan-Meier survival plot for fractures in different total dairy consumption groups of women. The four curves represent fracture survival probability in different total dairy consumption groups (crude data). The lowest fracture survival probability is shown by the group consuming ≥800 g/d total dairy.

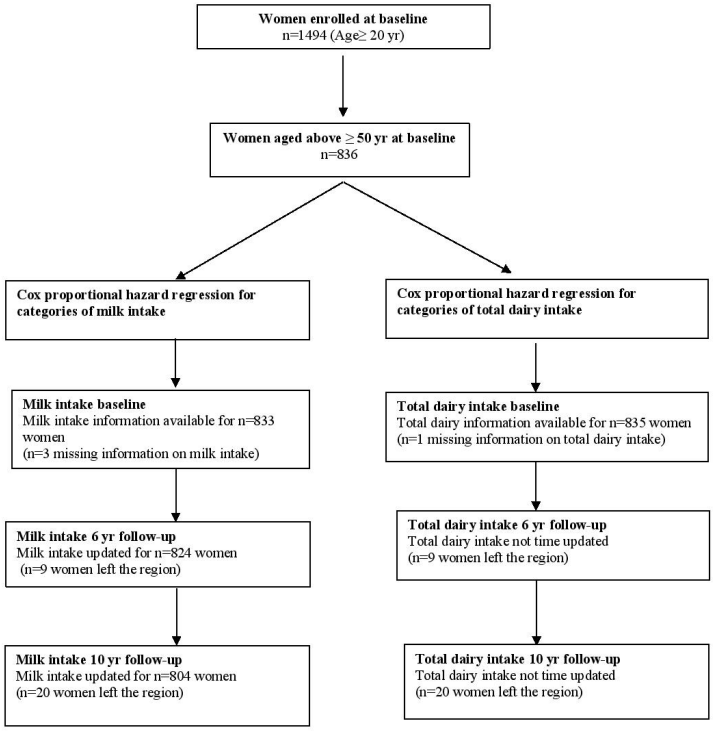

FIGURE 1
Participant flow chart. The figure represents the number of women at baseline, 6 and 10-year follow-up waves, and women left the region.

209x297mm (150 x 150 DPI)

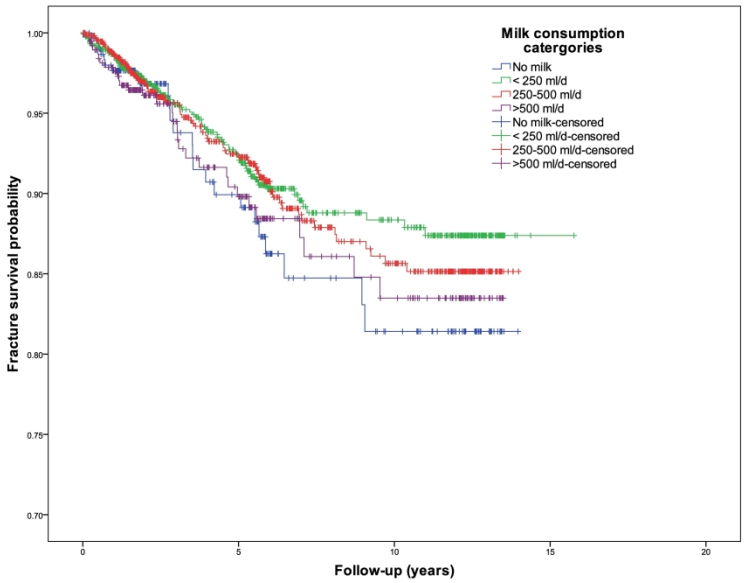

FIGURE 2
Kaplan-Meier survival plot for fractures in different milk consumption groups of women. The four curves represent fracture survival probability in different milk consumption groups (crude data). The lowest fracture survival probability is shown by the group consuming no milk.

352x211mm (300 x 300 DPI)

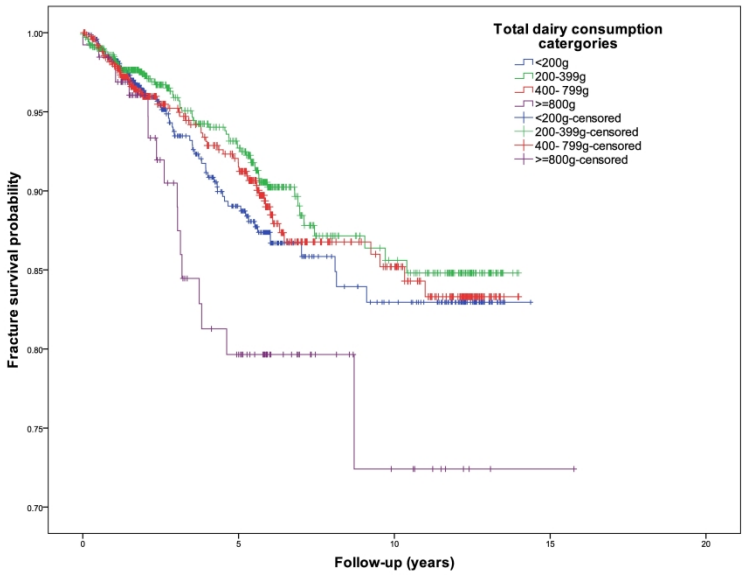

FIGURE 3
Kaplan-Meier survival plot for fractures in different total dairy consumption groups of women. The four curves represent fracture survival probability in different total dairy consumption groups (crude data). The lowest fracture survival probability is shown by the group consuming ≥800 g/d total dairy.

352x211mm (300 x 300 DPI)

Supplementary Table 1

Baseline characteristics of participants stratified by total dairy consumption categories[1]

	<200 g/d	200-399 g/d	400-799 g/d	> 800 g/d
Number of women	236	314	243	42
Age at entry, yr	69.1 (59.5-80.6)	70.4 (60.6-80.8)	69.5 (58.7-77.0)	72.5 (64.2-79.2)
Body mass index, kg/m^2	26.1 (23.2-29.7)	27.0 (23.7-30.4)	25.9 (23.6-29.7)	25.3 (23.5-27.1)
Milk, *n* (%)				
No milk	55 (26)	6 (2)	9 (4)	0 (0)
<250 mL/d	177 (76)	203 (65)	12 (5)	1 (2)
250-500 mL/d	2 (1)	105 (33)	178 (73)	1 (2)
>500 mL/d	0 (0)	0 (0)	44 (18)	40 (95)
Yogurt, g/d	0.0 (0.0-0.0)	0.0 (0.0-57.1)	28.6 (0.0-85.7)	85.7 (57.1-142.8)
Cheese, g/d	6.9(2.3-13.7)	9.1(4.6-16.0)	16.0 (8.9-31.4)	16.0 (8.0-35.4)
Ice-cream, g/d	0.0(0.0-7.7)	0.0(0.0-7.7)	1.0(0.0-11.6)	0.0 (0.0-7.7)
Bone mineral density, g/cm^2	0.809 ± 0.152	0.830 ± 0.162	0.843 ± 0.147	0.790 ± 0.132
Whole-body fat, kg	27.2 (20.3-33.1)	27.1 (20.3-33.1)	25.9 (20.8-32.7)	27.4 (27.4-27.4)
Lean mass, kg	36.8 ± 5.0	37.3 ± 4.4	37.4 ± 4.6	36.4 ± 4.0
Dietary calcium, *n* (%)				
<1000 mg/d	231(100)	310 (99)	157 (65)	0 (0)

≥1000 mg/d	0 (0)	3 (0.9)	86 (35.4)	41 (100)
Falls in the past, *n* (%)	43 (19)	68 (22)	49 (20)	11 (26)
Fractures in the past, *n* (%)	90 (38)	112 (36)	79 (33)	21 (50)
Incident cancer, *n* (%)	30 (13)	48 (15)	30 (12)	8 (19)
Diabetes, *n* (%)	17 (11)	25 (12)	23 (13)	4 (14)
Hypertension, *n* (%)	144 (61)	199 (63)	145 (60)	25 (60)
Smoking, *n* (%)				
Smokers	28 (13)	24 (8)	21 (9)	3 (7)
Non-smokers	208 (88)	290 (92)	222 (91)	39 (93)
Mobility, *n* (%)				
Highly active	109 (47)	152 (48)	134 (55)	20 (48)
Less active	125 (53)	162 (52)	109 (45)	22 (52)
Supplemental calcium, *n* (%)	34 (14)	40 (13)	30 (12)	12 (29)
Supplemental vitamin D, *n* (%)	30 (13)	37 (12)	23 (10)	10 (24)
Bisphosphonates, *n* (%)	1 (0.4)	3 (1)	4 (2)	0 (0)
Anabolic therapies, *n* (%)	0 (0)	0 (0)	0 (0)	0 (0)
HT, *n* (%)	37 (16)	49 (16)	36 (15)	4 (10)
IRSD *n* (%) / quintile				
1	37 (16)	71 (23)	42 (17)	5 (12)
2	48 (20)	73 (23)	48 (20)	12 (29)

3	64 (27)	61 (19)	57 (23)	10 (24)
4	39 (17)	55 (18)	36 (15)	9 (21)
5	48 (20)	54 (17)	60 (25)	6 (14)
Education, *n* (%)				
<12 years	205 (89)	275 (88)	207 (86)	35 (83)
≥12 years	26 (11)	37 (12)	34 (14)	7 (17)
Marital status, *n* (%)				
Living with partner	91 (39)	149 (48)	103 (42)	27 (64)
Living alone	143 (61)	165 (53)	140 (58)	15 (36)

[1]Data reported as mean± SD, median (IQR) or n (%); Total dairy comprises milk, cheese, yogurt and ice-cream

Reporting checklist for cohort study.

Based on the STROBE cohort guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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In your methods section, say that you used the STROBE cohortreporting guidelines, and cite them as:

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		Reporting Item	Page Number
Title and abstract			
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	1
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	#3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	#4	Present key elements of study design early in the paper	5
Setting	#5	Describe the setting, locations, and relevant dates, including periods of	5

		recruitment, exposure, follow-up, and data collection	
Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up.	5
Eligibility criteria	#6b	For matched studies, give matching criteria and number of exposed and unexposed	N/A
Variables	#7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6,7,8,9
Data sources / measurement	#8	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable.	6,7,8,9
Bias	#9	Describe any efforts to address potential sources of bias	16
Study size	#10	Explain how the study size was arrived at	17
Quantitative variables	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	10,11
Statistical methods	#12a	Describe all statistical methods, including those used to control for confounding	10,11
Statistical methods	#12b	Describe any methods used to examine subgroups and interactions	N/A
Statistical methods	#12c	Explain how missing data were addressed	6
Statistical methods	#12d	If applicable, explain how loss to follow-up was addressed	17
Statistical methods	#12e	Describe any sensitivity analyses	11
Results			
Participants	#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable.	Figure1

Participants	#13b	Give reasons for non-participation at each stage	Figure 1
Participants	#13c	Consider use of a flow diagram	Figure 1
Descriptive data	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable.	11/24
Descriptive data	#14b	Indicate number of participants with missing data for each variable of interest	N/A
Descriptive data	#14c	Summarise follow-up time (eg, average and total amount)	12/16
Outcome data	#15	Report numbers of outcome events or summary measures over time. Give information separately for exposed and unexposed groups if applicable.	
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	11/12
Main results	#16b	Report category boundaries when continuous variables were categorized	7
Main results	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	11
Discussion			
Key results	#18	Summarise key results with reference to study objectives	13
Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	17
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	18
Generalisability	#21	Discuss the generalisability (external validity) of the study results	18
Other Information			

Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	18

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1

Association Between Dairy Intake And Fracture In An Australian Based Cohort Of Women: A Prospective Study

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Abstract

Objective: Given the inconsistent evidence on dairy consumption and risk of fracture, we assessed the association between milk/total dairy consumption and major osteoporotic fracture (MOF) in women from the Geelong Osteoporosis Study (GOS).

Methods: Women aged ≥50yr (n= 833) were followed from baseline (1993-1997) to date of first fracture, death, or 31 December 2017, whichever occurred first. Dairy consumption was assessed by self-report at baseline and the follow-up phases. Major Osteoporotic fractures (MOFs) (hip, forearm, clinical spine and proximal humerus) were confirmed radiologically. Multivariable adjusted Cox proportional hazard models were used to determine associations between milk/total dairy (milk, cheese, yogurt, ice-cream) consumption and MOFs. Cross-sectional associations between milk/total dairy consumption and serum high sensitivity C-reactive protein (hsCRP), C-terminal telopeptide (CTx) and procollagen type 1 N-terminal propeptide (P1NP) at baseline were investigated using multivariable linear regression.

Results: During follow-up (11,507 person-years), 206 women had a MOF. Consuming >500 mL/d of milk was not significantly associated with increased HR for MOF. Non-milk (1.56; 95% CI 0.99, 2.46) drinkers and consumption of ≥800 g/d total dairy (1.70; 95% CI 0.99, 2.93) had marginally higher hazard ratio (HR) for MOF compared to consuming <250mL/d of milk and 200-399g/d of total dairy respectively. Milk consumption was inversely associated with serum hsCRP and CTx but total dairy consumption was not associated with these serum markers.

Conclusion: Higher milk consumption did not increase the risk for MOF in older women. However, a trend for increased MOF were detected in zero milk and higher total dairy consuming women.

Key words: Fractures, Milk, Osteoporosis, Dairy, Inflammation

Strengths and limitations of the study

⇒ Although this study contained a modest sample size, it replicated the findings of previous studies.

⇒ Random sample selection from the general population is a strength of the study

⇒ The prospective study design strengthens the outcomes of the study despite methodological inconsistencies in capturing dietary data.

⇒ As data for total dairy consumption were assessed at baseline only, we cannot account for dietary changes during follow-up and this limits the interpretation of the longitudinal analysis of the association between total dairy consumption and the risk for major osteoporotic fracture

⇒ The conclusions of this study cannot be generalised to a broader population as this study was focused on a cohort of women.

Introduction

Osteoporosis is a chronic multifactorial disease that is defined as low bone mass and impaired bone micro-architecture (1, 2). The presence of osteoporosis substantially increases the risk of sustaining a fracture, especially at the hip, spine, forearm and proximal humerus, which are known as the major osteoporotic fracture (MOF) sites. However, the largest absolute number of fractures occurs in people with a moderate deficit in bone density (osteopenia) (3, 4). Falls risk, which can be affected by factors such as medication use, mobility level and environmental hazards is also an important consideration, as most fractures are preceded by a fall (5-8). Of the factors (e.g. genetics (9, 10), age (11, 12), lifestyle habits (2), sex (13)) influencing fractures, nutrition plays a substantial role in the aetiology of osteoporosis (14, 15). Adequate calcium and protein intakes are necessary in order to maintain skeletal integrity and strength (16, 17). Milk/dairy products are key components in the western diet and contain a myriad of nutritional components (calcium, vitamins and proteins) and a majority of an individual's dietary calcium needs are fulfilled by intake of dairy products (18, 19). Additionally, milk/dairy products have been widely recommended to osteoporosis patients by clinicians and healthcare professionals considering the beneficial effects associated with dairy consumption (20, 21).

However, data regarding milk consumption as a strategy for fracture prevention has shown inconclusive results. Findings from large Swedish cohorts reported that women who consumed three or more glasses of milk per day had higher risk for any fractures while fermented dairy consumption was inversely associated with fractures (22). However, Feskanich *et al.* have shown in two large US cohorts that each serving of milk per day was associated with an 8% reduction of risk for hip fracture, whereas total dairy intake was associated with a 6% reduction of risk for hip fractures in men and women combined (23). Holvik *et al*. found no association between increased milk intake and risk for hip fractures in Norwegian women and men (24). The most recently published meta-analysis (2018), which included 18 observational studies,

showed that higher milk intakes were not associated with fractures in both sexes combined (25). However, it is worth noting that there was a large amount of heterogeneity between studies in terms of reporting milk/dairy intake, number of fractures, use of different confounders for adjustment and fracture ascertainment methods.

Although the overall evidence on increased milk intake appears supportive of reducing fractures, dissecting milk further to the molecular level demonstrates that milk contains compounds such as D-galactose (milk sugar) and A1-beta-casein (mutated protein variant) that may be detrimental to bone health (26-28). Pre-clinical studies show that these compounds are implicated in inflammation and oxidative stress pathways that can negatively impact bone metabolism (29, 30). Moreover, Pasco *et al.* previously indicated that increased milk intake is associated with depressive disorder (31), a condition that is comorbid with fractures (32, 33). Therefore, we hypothesised that increased milk consumption may be associated with increased risk for MOF by triggering inflammation and oxidative stress.

Other milk derived products such as yogurt and cheese have a distinct biological profile to milk and may have a protective role in bone health due to the presence of probiotics, prebiotics and other bioactive compounds; these in turn have the potential to attenuate inflammation and oxidative stress (34, 35). Studies have assessed the effects of these products on bone separately to milk; however, the synergistic impact of dairy products (including milk, yogurt, cheese, ice-cream) with different molecular and biological profiles is poorly unravelled. Therefore, we aimed to assess the association between total dairy consumption and MOF in women. We also investigated potential mechanisms by which milk/total dairy may mediate the risk for MOF. For this purpose, the cross sectional association between milk/total dairy consumtion, and serum high sensitivity C-reactive protein (hsCRP), C-terminal telopeptide (CTx) and procollagen type 1 N-terminal propeptide (P1NP) were examined at baseline.

Methods

Patient and Public involvement

Patients were not involved in the planning and design of the study.

Study Population

This study used data from the Geelong Osteoporosis Study (GOS), a large population-based cohort study based in south-eastern Australia. Inclusion criteria were: living in the Barwon Statistical Division (BSD) for > 6 months and able to provide written informed consent. Women in the BSD were selected at random from the electoral roll during the years 1993-1997 to participate in the study (36). An age-stratified sample of 1,494 women was enrolled in the study with a participation of 77.1%. Subsequent assessments for these women commenced in 1995, 1998, 2000, 2002, and 2004, referred to as 2-year, 4-year, 6-year, 8-year, and 10-year follow-up phases. The cohort profile is explained elsewhere (36). For the purposes of the analysis women only ≥50yr at baseline were considered. Of the 836 women aged ≥ 50 yr, 833 women were included in the analysis after excluding records with missing information on milk intake (Figure1). Study participants provided written informed consent. The study was approved by the Human Research Ethics Committee at Barwon Health.

Outcome Measures

Post-baseline incident fractures were identified using a method that have been validated for fracture ascertainment in the region. Radiological reports (X-ray) of fractures from all radiological centres in the region were scrutinised to identify and confirm fractures. (37, 38). Trained research personnel examined each record individually and determined the most appropriate international code of diseases version 9 (ICD-9) codes for fracture site, as well as level of trauma (39). MOFs were defined as fractures at the hip, forearm, clinical spine and

proximal humerus, according to the fracture risk assessment tool (FRAX) developed by the University of Sheffield for clinical use (40). Pathological and high trauma fractures were excluded. Information on death was collected from the National Deaths Index (Australian Institute for Health and Welfare).

Dairy consumption and diet

Information on dairy was available at baseline, 6 year and 10-year follow-up. At baseline and 6-year follow-up, dietary information was documented by a self-reported questionnaire that contained questions on 35 foods and beverages on average. Participants were asked questions about the habitual/type of (all forms e.g. milk used in cooking, baking and in coffee) milk consumed (whole, reduced fat, calcium fortified, soy, goat's milk, butter milk, and evaporated) and the quantity consumed each day. In the questionnaire, it was stated that one cup of milk is considered equivalent to 250 mL. Therefore, participants chose the type and quantity of milk consumed from any pre-determined milk categories and only cow's milk was considered (none, < 125 mL (< ½ cup), 125 -249 mL (½-<1 cup), 250-499 mL (1-<2 cups), 500- 999 mL (2-<4 cups), ≥ 1000 mL (≥ 4 cups) per day). The lowest response categories <125 mL/d, 124-249 mL/d were collapsed into one category indicating "< 250 mL/d" and the highest response categories, 500- 999 mL/d, ≥ 1000 mL were combined into one category indicating "> 500 mL/d"; this was due to low proportions responding to the lower and higher categories and for the compatibility with the 10-year follow-up dietary data. The second lowest category was chosen as reference for milk consumption as this category benefits robustness due to higher number of participants within the category.

Information on other dairy products such as cheese, yogurt (all forms e.g. cheese, and yogurt used in cooking, baking) and ice-cream consumption were also documented using this self-reported questionnaire. Participants were specifically asked about different types of cheese they

consumed on a weekly basis including hard cheese e.g. cheddar, tasty (servings/week; 1 serving = 16 g); soft cheese e.g. cream, cottage (servings/week; 1 serving= 20 g); and fruche (servings/week; 1 serving = 100 g). Fruche is a form of soft cheese (fromage frais) and thus was categorised as cheese. Total cheese consumption was converted to grams consumed per day (g/d). Yogurt (servings/week; 1 serving= 200 g) and ice-cream consumption (servings/week; 1 serving = 27 g) were reported as servings per week and this was converted to grams consumed per day. Daily total dairy consumption was calculated at baseline by combining values for cow's milk, all forms of cheese, yogurt and ice-cream consumed and was expressed in grams consumed per day. The clustered nature of total dairy distribution, made it unfeasible to consider it as a continuous variable for analytical purpose, and as such it was treated as categorical variable in the analysis and categorised as < 200 g/d, 200-399 g/d, 400-700 g/d, ≥800 g/d. The second lowest category was chosen as reference for total dairy because it was the largest group.

At 10-year follow-up, information on milk/dairy consumption was collected using a validated food frequency questionnaire. The Cancer Council Victoria Dietary Questionnaire captures information on 74 foods and six alcoholic beverages over the previous 12 months and is validated for assessing habitual dietary intake in Australian women (41). Participants were queried on their usual type (none, full cream, reduced fat, skim, and soymilk) and quantity of milk consumed on a daily basis. Participants were advised that 1 cup of milk is equivalent to 250 mL of milk. Furthermore, participants indicated their daily milk intake by selecting from pre-determined categories of milk intakes and only cow's milk was considered (none, < 250 mL (<1cup), 250- 499 mL (1-<2 cups), 500- 750 mL (2-3 cups) and > 750 mL (> 3 cups) per day). The highest response categories, 500-750 mL/d, > 750 mL/d were combined as to one category indicating "> 500 mL/d"; this was due to low proportions responding to the higher

categories. This questionnaire also captured information on cheese, yogurt and ice-cream intake of participants.

A separate calcium-specific dietary questionnaire was used to capture information on dietary calcium intake. This questionnaire included information on a range of common calcium-dense food sources, which allowed calculation of dietary calcium intakes in mg per day (mg/d) and validated against 4-day weighed food intakes (19). Dietary calcium intake was categorised into two strata (< 1000 mg/d, ≥ 1000 mg/d).

Other information and potential confounders

All measurements were assessed at the baseline visit. Weight and height were recorded to the nearest 0.1 kg and 0.1 cm respectively and body mass index (BMI) calculated as weight/height^2 (kg/m^2). Dual-energy x-ray absorptiometry (DXA; Lunar DPX-L; Lunar, Madison, WI) was performed to evaluate bone mineral density (BMD; g/cm^2) at the femoral neck, and whole-body fat (kg), and 'lean' mass (kg) which represents the water and protein content in muscle, skin, connective tissue and lean component in adipose tissue.

Self-report questionnaires were used to obtain information on mobility, physical activity levels, smoking status, medications, prior falls, and fractures. Participants were asked to select their mobility level from pre-determined 7-point scale (very active, active, sedentary, limited, inactive, chair or bed ridden, bedfast - examples were given in the questionnaire to assist the participant to choose the most suitable option). These categories were further condensed to two groups, highly active and less active, for the purpose of this analysis. Physical activity level was also assessed from questions regarding work/home and recreational/sports, on a 3 point-scale which provided options for participants to select from moderate, hard and very hard. Participants were also asked to enter the time spent on each activity level on a weekly basis.

Information on current smoking status was categorised as smoking or non-smoking. Use of medications that positively or negatively influence bone included bisphosphonates, anabolic therapies, hormonal replacement therapies (HT), and oral glucocorticoids. Participants were asked to list the use of supplements and this information was used to assess the calcium and vitamin D supplementation usage. Use of supplementary calcium and vitamin D were documented at baseline, 6 yr and 10 yr follow-up.

The definition of falls (when you suddenly find yourself on the ground, without intending to get there, after you were in either a lying, sitting or standing position) was explicit in the questionnaire and asked participants whether or not they experienced a similar scenario over the past 12 months. Information regarding previous fractures and cancer diagnoses was also captured by self-reported questionnaires. An automated device (Takeda Medical UA-751) was used to measure blood pressure in a sitting position. Women were considered hypertensive if they had a systolic blood pressure over 140 mmHg and/or a diastolic pressure above 90 mmHg and/or use of antihypertensive medication in the presence of self-reported hypertension. Women were identified as having diabetes if they had a fasting plasma glucose ≥ 7.0 mmol/L (126 mg/dL), self-reported diabetes and/or use of antihyperglycemic agents.

Information pertinent to educational qualifications were gathered on a 7-point scale: never attended school, primary school, some secondary school, completed secondary school, post-secondary qualifications, university or other tertiary qualifications, and can't remember. These categories were compressed to education received for less than 12 years or more than 12 years for the purpose of this analysis. Information on marital status was dichotomised as living alone or living with a partner. The socio-economic status of the cohort participants was measured by the Index of Relative Socioeconomic Disadvantage (IRSD), an area-based index that measures relative disadvantage of socio-economic status. This tool imputes a span of information on

economic and social conditions of people and household within an area and is represented in quintiles. The most disadvantaged category is indexed by quintile 1 (42).

Biomarkers

At baseline, venous blood was collected after an overnight fast and stored at -80 ^{0}C until batch analysis. Markers of bone turnover, serum C-terminal telopeptide (CTx), a marker of bone resorption, and serum procollagen type 1 N-terminal propeptide (P1NP), a marker of bone formation, were analysed from the blood samples. In addition, high sensitivity serum C-reactive protein (hsCRP), a marker of systemic inflammation, was determined from the blood samples. Serum hsCRP was measured by the Roche immunoturbidometric 'CRP" and 'C-reactive protein (latex) high sensitivity methods. Details of these analytical methods have been described elsewhere (43, 44).

Statistical analysis

Characteristics of participants were described by mean (± SD) or median (IQR) or relative frequencies (%) stratified by milk consumption categories (no milk, <250 mL/d, 250-500 mL/d, > 500 mL/d). Participant characteristics across categories of milk consumption were compared using one-way ANOVA or Kruskal-Wallis-H test for continuous data and Chi-square test (or Fisher's exact test) for categorical data. The null hypothesis was rejected at an α level of 0.05 and a post hoc multiple comparison was performed using Bonferroni corrections. Additionally, participant characteristics were described based on total dairy categories (<200 g/d, 200-399 g/d, 400-799g/d, ≥ 800 g/d) (Supplementary Table 1).

Cohort participants were followed from their baseline appointment to date of first fracture, death, or 31 December 2017. Cox proportional hazard regression was used to estimate age adjusted hazard ratio (HR) and their 95% confidence intervals for categories of milk consumption (no milk, < 250 mL/d, 250-500 mL/d, > 500 mL/d).

Covariates (BMD, BMI, smoking, alcohol consumption, pre-baseline fractures incidents, diabetes, IRSD, education, mobility, medications that influence bone metabolism, calcium and vitamin D supplements) were assessed in bivariate Cox regression analysis to determine their impacts on the association between milk/total dairy consumption and fractures. The covariates that impacted the hazard ratio when added or removed (considering the statistical significance and change of HR in the exposure of interest) from the model were included in the final Cox regression model. In addition, when deciding on the confounders, the potential of the covariate to be associated with both the exposure and outcome was also considered. The final model consisted, age, oral glucocorticoid use, HT use and pre-baseline factures as confounders. Information on milk consumption, oral glucocorticoid use, and HT use were time updated at the 6 and 10-year follow-up. Age was time updated in all follow-up waves. Information on pre-baseline fractures were not time updated and kept constant for the analysis. We also performed a multivariable adjusted sensitivity analysis using baseline milk values only.

In addition, a Cox proportional hazard regression was also used to estimate the age adjusted HR and their 95% confidence intervals for total dairy consumption categories (< 200 g/d, 200-399 g/d, 400-799 g/d, ≥ 800 g/d). Selection of potential confounders was performed according to the aforementioned method. The final model consisted age, oral glucocorticoid use, HT use, and prior fractures as confounders. For this analysis total dairy consumption was not time updated due to the inconsistent dietary tools used in capturing information on dairy products during the follow-up waves; therefore, baseline only values for total dairy consumption was used in the analysis. Oral glucocorticoid use and HT use were time updated at the 6 and 10-year follow-up; age was time updated at all follow-up waves; pre-baseline fractures were not time updated and kept constant for the analysis.

The proportional hazard assumptions were confirmed graphically by log(-log(survival)) plots for both daily milk and total dairy consumption. Time to first fracture (survival) curves were illustrated using Kaplan-Meier estimator of the survival function using product limit estimator.

The cross-sectional associations between milk/total dairy consumption and serum markers of inflammation (hsCRP) and bone turnover (CTx, P1NP) were assessed using multivariable linear regression models at baseline with potential confounders. Women who had missing information (n=45) on inflammatory and bone turnover markers were excluded from the analysis. Serum markers of inflammation and bone turnover were log transformed due to the skewed nature of data. For all analyses, STATA 15 and SPSS 25 was used.

Results

Descriptive characteristics of the cohort stratified by milk consumption categories are presented in Table 1. Of 833 women, 8.4% (*n*=70) did not consume milk and 47.2% (*n*=393), 34.3% (*n*=286) and 10.0% (*n*=84) consumed < 250 mL/d, 250-500 mL/d and > 500 mL/d of milk, respectively. There was no difference observed in women's median age among the four-milk consumption categories. Women who consumed >500 mL/d of milk reported the highest cheese intake. The group that consumed < 250 mL/d of milk had the highest proportion of women reporting < 1000 mg/d of dietary calcium intake. On the other hand, the group that consumed > 500 mL/d of milk had the highest proportion of women reporting ≥1000 mg/d of dietary calcium intake (Table 1). The proportion of women consuming supplementary calcium and vitamin D was high among the non-milk consumers. There were no differences detected for other parameters across the four-milk consuming groups.

During 11,507-person years of follow-up, 206 women sustained a MOF (spine=96; humerus=14; wrist=51; hip=45) and 503 women died. Women who consumed no milk reported the highest fracture rate (Table 2) and the crude fracture survival probability curve also showed

that women who consumed no milk had the lowest survival probability for fractures (Figure 2). Concordantly, women who reported no milk consumption showed marginally significant higher age adjusted (1.54, 95% CI 0.98, 2.44, P= 0.06) and multivariable adjusted (1.56, 95% CI 0.99, 2.46, *P*=0.06) HR for MOF compared to women who consumed < 250 mL/d of milk. The unadjusted (1.28, 95% CI 0.84,1.96, *P*=0.25), age adjusted (1.23, 95% CI 0.80,1.88, *P*=0.34), and multivariable adjusted (1.15, 95% CI 0.75,1.75, *p*=0.53) HR for MOF were not significantly higher in women who consumed > 500 mL/d of milk compared to women who consumed < 250 mL/d of milk. The multivariable adjusted sensitivity analysis, which was performed using baseline milk values only resulted a non-significant higher HR ratio for non-milk consumers (HR:1.53; CI: 0.96-2.44; *p*=0.07) and >500 mL/d of milk consumers (HR:1.13; CI:0.74-1.72; *p*=0.58) compared to consuming < 250 mL/d milk of milk.

When total dairy consumption was considered, women who consumed more than ≥ 800 g/d demonstrated the highest fracture rate (Table 3). This was also confirmed by the crude fracture survival probability curve, which indicated the lowest survival probability for fractures in women who consumed ≥ 800 g/d total dairy (Figure 3). Consistently, women who consumed ≥ 800g/d total dairy showed higher age adjusted (2.01, 95%CI 1.88, 3.44, *P*=0.01) and multivariable adjusted (1.70, 95% CI 0.99, 2.93, *P*=0.05) HR for MOF compared to women who consumed 200-399 g/d of total dairy (Table 3).

An inverse association was observed between milk consumption and serum markers of inflammation (hsCRP) and serum markers of bone resorption (CTx); women who consumed > 500 mL/d of milk had the lowest concentrations of serum hsCRP (-0.45; 95%CI: -0.82, -0.07; *P*= 0.02) and serum CTx (-0.25; 95% CI: -0.48, -0.02; *P*= 0.03) (Table 4). No association was found between milk consumption and serum marker of bone formation (P1NP). Moreover, there was no association found between total dairy consumption categories and serum hsCRP, CTx and P1NP (Table 4).

Discussion

In our study of older Australian women, we detected no significant association between higher milk consumption (> 500 mL/d) and increased risk for MOF. However, we found that zero milk and higher total dairy (≥ 800 g/d) consumptions had marginally higher risk for MOF.

Acquiring the daily recommend calcium through diet/supplements is considered the easiest and safest lifestyle modification that could be achieved as a part of prevention and management of osteoporosis (45). Milk/dairy products are considered the ideal source of calcium that consumed in recommended quantities, may approximately satisfy the daily calcium requirements (46, 47). In general, 1200 mg/d of calcium is recommended for women aged > 50yr (48) and potentially four serves of milk (1 serve = 250 mL = 300 mg of calcium) can cover this need. Our study results were in support of this suggesting that not consuming milk may increase the risk of MOF.

However, some components in milk such as D-galactose (49) and A1-beta-casein, (50) are believed to possibly mediate the unfavourable consequences associated with milk consumption. D-galactose has proven to be involved in the ageing process in mice, which encompassed series of events such as oxidative stress and chronic inflammation (26). Besides, existing epidemiological data show that some negative health consequences (Ischemic heart disease and Type 1 diabetes) associated with milk consumption may be due to the A1 beta-casein fraction in milk (51-55). However, robust evidence from clinical trials are lacking to confirm causality.

The Swedish cohort study speculated that increased milk intake may be deleterious to bone due to the D-galactose content in milk, and showed that women who consumed more than 3 glasses of milk compared to 1 glass of milk per day had higher risk for any fractures and mortality (22). Additionally, a positive correlation between milk consumption and both oxidative stress

marker in urine (8-iso-PGF2α) and inflammatory marker in serum (IL-6) were detected in the Swedish cohort (22). Hence, those findings offered support to the hypothesis that increased milk intakes are deleterious to bone and this may be mediated through D-galactose in milk (22). However, many other studies (24, 25, 56) including our study did not find any evidence to show that increased milk consumption is associated with fractures. We also assessed the association between total dairy consumption (milk, cheese, yogurt and ice-cream) and MOF. Here, we found that women who consumed ≥ 800 g/d of total dairy showed higher risk for MOF compared to women consuming moderate levels.

This study also attempted to assess the underpinning mechanisms by, which higher milk/total dairy products may instigate fractures. It was expected that increased milk/total dairy intakes may augment systemic inflammation; thereby negatively influence bone metabolism, and increase bone fragility and risk for fractures (44, 57). Serum hsCRP is deemed a sensitive marker of systematic inflammation and higher concentration of serum hsCRP has been detected in inflammatory diseases and also associated with fractures(58, 59). Therefore, the cross-sectional association between milk/total dairy categories and serum marker of inflammation (hsCRP) was tested. The lowest serum hsCRP concentration was detected in women who consumed > 500 mL/d of milk. Our findings did not support our hypothesis and they were corroborated by other literature that showed decreased CRP levels with increased milk/dairy intake (60). Also, we did not find any association between total dairy consumption and serum hsCRP. We also, assessed whether there is an association between milk/total dairy consumption categories and markers of bone turnover. There were no clear patterns of associations found between milk consumption and serum marker of bone formation (P1NP). But women consuming >500 mL/d of milk had the lowest concentrations of serum marker of bone resorption (CTx). Moreover, there was no association detected between total dairy consumption and serum CTx and P1NP.

Our study has several strengths. One strength of the study is, GOS comprise a randomly selected group of participants, which has shown to be similar to the broader Australian population (in terms of income, SES, etc.). However, our findings are not generalisable to men, nor other countries with different distributions of ethnicities, diet patterns and other factors. However, our study results are likely generalisable to the broader Australian population of women. We were able to perform a longitudinal analysis that incorporated a long follow-up time with a median of 14.26 years as GOS is a cohort study. Additionally, data on the main exposure variable and confounders were updated several times during the period of follow-up, which enhanced the robustness of our analyses. Cognisant that osteoporosis is a multifactorial disease, we included many possible potential confounders (age, oral glucocorticoids, HT, and past fractures) in the analysis. Also, we used an objective method of ascertaining/confirming incident fractures from radiological reports rather than relying on self-reported information. However, this was a regionally validated fracture ascertainment method, which may not account for fractures in participants who left the region (Figure 1).

However, our study did have some limitations. We were unable to describe the association between milk/total dairy intake and fracture risk, as U/J shaped graphs showing higher risks in the zero and high consumption groups and the lowest risk in the low-intermediate consumption groups because of the low number of fractures, which may lead to lower precision in the estimates. The study sample size was modest. A post-hoc power calculation showed that based on annual fracture rate of 14.10 per 1,000 in the reference group (<250 mL/d milk consumption) the minimum detectable effect size (i.e. RR) ranged from 1.5 to 1.9, which was bigger than observed risk ratios from unadjusted and adjusted Cox models. Although the dietary questionnaire was designed to provide information on participants' habitual dairy intake, it is possible that dairy contained in manufactured/prepared products is not captured and thereby it underestimates total dairy consumption. In addition, when querying about the

type of milk consumed, A2 milk/milk products (which contains exclusively A2 milk proteins) were not provided as an option to be selected by the participant; thus, we were not able to investigate particular milk proteins as potential mediators in the association with milk consumption. Also, total dairy consumption in the Cox regression analysis, was not time updated as dietary information was not collected consistently across all follow-up visits and we thus performed an analysis using the baseline data only. This might have led to unaccounted changes in exposure status that may have occurred during the period of follow-up. In addition, some participants were lost to follow-up during the study due to leaving the region (n=29), which prevented time-dependent updates on their information. As with all observational/follow-up studies, attrition is unavoidable. In the interim, there may have been other unrecognised confounding in our study.

Conclusion

Taken together, our study results suggest that higher milk consumption is not associated with increased risk for MOF; however, zero milk consumption appears to be associated with an increased risk for MOF. Also, higher consumption of total dairy (milk, yogurt, cheese and ice-cream) may increase the risk for MOF, indicating a negative influence on bone health. Further studies are warranted to identify optimal levels of milk and total dairy consumption ranges and the potential mechanisms by which total dairy consumption may influence the risk for fracture.

Contribution: HA, KLH-K, MM, FNJ, JAP contributed to the interpretation of data, and critical appraisal of the manuscript and HA constructed the manuscript.

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Patient consent for publication: not required.

Ethical approval: The study was approved by the Human Research Ethics Committee at Barwon Health.

Data sharing statement: Data for this study will be available upon request. Request can be sent to gos@barwonhealth.org.au

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TABLE 1

Baseline characteristics of participants stratified by milk consumption categories[1]

	No milk	< 250 ml/d	250-500 ml/d	> 500 ml/d
Number of women	70	393	286	84
Age at entry, yr	68.2 (58.2-77.6)	69.1 (59.2-80.3)	71.4 (60.5-80.4)	71.7 (64.2-80.4)
Body mass index, kg/m^2	25.1 (22.1-28.6)	26.8 (24.1-30.3)	25.9 (23.5-29.9)	25.3 (23.2-28.9)
Yogurt, g/d	0.0 (0.0-57.1)	0.0 (0.0-57.1)	3.6 (0.0-57.1)	0.0 (0.0-85.7)
Cheese, g/d	9.1 (3.4-22.9)	9.1 (4.6-16.0)	11.0 (4.6-22.9)	**13.7 (6.9-25.1)
Ice-cream, g/d	0.0 (0.0-11.6)	0.0 (0.0-7.7)	0.0 (0.0-7.7)	0.0 (0.0-11.6)
Bone mineral density, g/cm^2	0.792 ± 0.163	0.830 ± 0.156	0.832 ± 0.146	0.808 ± 0.161
Whole-body fat, kg	24.1 (18.9-32.2)	27.6 (20.7-34.0)	25.7 (20.5-32.6)	24.6 (19.5-29.0)
Lean mass, kg	36.3 ± 4.8	37.3 ± 4.7	37.3 ± 4.6	36.9 ± 4.1
Dietary calcium, *n* (%)				
<1000 mg/d	65 (93)	**386 (98)	246 (86)	1(1)
≥1000 mg/d	5 (7)	4 (1)	39 (13)	**82 (98)
Falls in the past, *n* (%)	14 (20)	73 (19)	63 (20)	21 (25)
Pre-baseline fractures, *n* (%)	24 (34)	146 (37)	93 (33)	39 (46)
Incident cancer, *n* (%)	7 (10)	58 (15)	37 (13)	14 (17)

Diabetes, *n* (%)	6 (9)	30 (8)	22 (8)	11 (13)
Hypertension, *n* (%)	47 (67)	242 (62)	172 (60)	51 (61)
Smoking, *n* (%)				
Smokers	64 (91)	348 (89)	267 (93)	78 (93)
Non-smokers	6 (9)	45 (11)	19 (7)	6 (7)
Mobility, *n* (%)				
Highly active	38 (54)	192 (49)	142 (50)	43 (51)
Less active	32 (46)	201 (51)	144 (50)	41 (49)
Supplemental calcium, *n* (%)	**16 (22)	49 (12)	34 (12)	17 (20)
Supplemental vitamin D, *n* (%)	**15 (21)	45 (11)	25 (9)	15 (18)
Bisphosphonates, *n* (%)	1 (1)	1 (0)	6 (2)	0 (0)
Anabolic therapies, *n* (%)	0 (0)	0 (0)	0 (0)	0 (0)
HT, *n* (%)	10 (14)	70 (18)	38 (13)	8 (10)
Oral glucocorticoids, *n* (%)	2 (3)	9 (3)	5 (2)	6 (7)
IRSD *n* (%) / quintile				
1	11(15)	81(21)	48 (17)	15 (18)
2	18 (26)	81(21)	63 (22)	19 (24)
3	16 (23)	97 (25)	58 (20)	20 (24)

4	12 (17)	58 (14)	55 (19)	14 (17)
5	13 (19)	76 (19)	62 (22)	16 (19)
Education, *n* (%)				
<12 years	63 (90)	342 (87)	248 (87)	69 (82)
≥12 years	7 (10)	46 (11)	37 (13)	14 (17)
Marital status, *n* (%)				
Living with partner	28 (40)	162 (41)	137 (48)	43 (51)
Living alone	42(60)	231(59)	149 (52)	41 (49)

[1] Data reported as mean± SD, median (IQR) or n (%); Milk comprises skim, low fat, full fat with a serving size of 1 cup = 250 mL;
**P< 0.01 Bonferroni corrected; HT=hormonal replacement therapy, IRSD=Index of Relative Socioeconomic Disadvantage
The most disadvantaged category is indexed by quintile 1

TABLE 2

Incident fracture rates (n/1000), unadjusted, age-adjusted, and multivariable adjusted HR for MOF in different milk consumption categories with their 95% confidence interval[1]

	Categories of milk consumption[1]			
	No milk	<250 mL/d	250-500 mL/d	>500 mL/d
Number of fractures (n)	24	82	71	29
Person years	1040.0	5001.0	4092.0	1373.4
Rate (n/1000)[2]	23.09	16.40	17.35	21.12
Unadjusted HR	1.40 (0.89, 2.21)[3]	1.00 (reference)	1.05 (0.76, 1.44)	1.28 (0.84, 1.96)
Age adjusted HR	1.54 (0.98, 2.44)	1.0 (reference)	1.00 (0.73, 1.37)	1.23 (0.80, 1.88)
Multivariable adjusted HR[4]	1.56 (0.99, 2.46)	1.0 (reference)	1.02 (0.74, 1.40)	1.15 (0.75, 1.75)

[1] Milk comprises skim, low fat, full fat with a serving size of 1 cup = 250 mL (time updated at 6 year and 10year follow-up waves)

[2] Fracture rates: number of cases per 1000-person years at risk

[3] 95% CI in parentheses (all such values)

[4] Adjusted for oral glucocorticoids, HT, (time updated at 6, 10-year follow-up waves), age (time updated at all follow-up waves) pre-baseline fractures (baseline values)

HR= hazard ratio, HT= hormonal replacement therapies, MOF=major osteoporotic fracture (fractures in hip, forearm, clinical spine and proximal humerus)

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TABLE 3

Incident fracture rates (n/1000), unadjusted, age-adjusted and multivariable HR for MOF in different total dairy products consumption categories with their 95% confidence interval[1]

	Categories of total dairy consumption[1]			
	<200 g/d	200-399 g/d	400-799 g/d	≥800 g/d
Fractures	61	66	62	17
Person years	3125.0	4362.1	3492.1	528.1
Rate (per 1000)[2]	19.52	15.13	17.75	32.19
Unadjusted HR	1.30 (0.91, 1.83)[3]	1.00 (reference)	1.18 (0.84, 1.68)	2.10 (1.23, 3.58)
Age adjusted	1.42 (1.00, 2.01)	1.00 (reference)	1.34 (0.94, 1.90)	**2.01 (1.18, 3.44)
Multivariable adjusted HR[4]	1.40 (0.98, 1.97)	1.00 (reference)	1.35 (0.95, 1.91)	1.70 (0.99, 2.93)

[1] Total dairy includes milk, cheese, yogurt and ice-cream

[2] Fracture rates: number of cases per 1000-person years at risk

[3] 95% CI in parentheses (all such values)

[4] Adjusted for oral glucocorticoids, HT, (time updated at 6, 10year follow-up waves), age (time updated at all follow-up waves) pre-baseline fractures (baseline values)

HR= hazard ratio, HT=hormonal replacement therapies, MOF=major osteoporotic fracture (fractures in hip, forearm, clinical spine and proximal humerus)

**p <0.05

TABLE 4

Association between milk/total dairy consumption categories, and serum markers of systemic inflammation and bone turnover with their 95% confidence interval [1]

	hsCRP[2] (mg/L)		CTx[3](ng/L)		P1NP[4](µg/L)	
	Coefficient	95% CI	Coefficient	95% CI	Coefficient	95% CI
Milk consumption categories[5]						
No milk	Reference		Reference		Reference	
< 250 mL/d	-0.29	-0.59, 0.01	-0.15	-0.33, 0.04	-0.10	-0.26, 0.06
250-500 mL/d	**-0.39	-0.70, -0.09	**-0.20	-0.39, -0.02	-0.05	-0.21, 0.11
>500 mL/d	**-0.45	-0.82, -0.07	**-0.25	-0.48, -0.02	-0.13	-0.33, 0.08
Total dairy consumption categories[6]						
< 200 g/d	Reference		Reference		Reference	
200- 399 g/d	0.06	-0.26, 0.15	-0.10	-0.22, 0.03	-0.08	-0.19, 0.02
400- 799 g/d	-0.17	-0.39, 0.04	-0.11	-0.24, 0.01	-0.03	-0.14, 0.10
≥800 g/d	-0.04	-0.44, 0.35	-0.15	-0.39, 0.09	-0.05	-0.27, 0.18

[1] Multivariable linear regression performed on baseline data (cross sectional) of 788 women aged ≥50yr; serum marker of systemic inflammation (hsCRP) and bone turnover (CTx-bone resorption: P1NP-bone formation) are log transformed

[2] Model adjusted for BMI, mobility, diabetes, oral glucocorticoids, hypertension

[3] Model adjusted for BMI, age, bisphosphonate, HT

[4] Model adjusted for age, HT, diabetes

[5] Milk comprises skim, low fat, full fat with a serving size of 1 cup = 250 mL

[6] Total dairy includes milk, cheese, yogurt and ice-cream

** P< 0.05.

BMI=body mass index, CTx=C-terminal telopeptide, hsCRP=high sensitivity C-reactive protein, HT=hormonal replacement therapies, P1NP=procollagen type 1 N-terminal propeptide

FIGURE 1

Participant flow chart. The figure represents the number of women at baseline, 6 and 10-year follow-up waves, and women left the region.

FIGURE 2

Kaplan-Meier survival plot for fractures in different milk consumption groups of women. The four curves represent fracture survival probability in different milk consumption groups (crude data). The lowest fracture survival probability is shown by the group consuming no milk.

FIGURE 3

Kaplan-Meier survival plot for fractures in different total dairy consumption groups of women. The four curves represent fracture survival probability in different total dairy consumption groups (crude data). The lowest fracture survival probability is shown by the group consuming ≥800 g/d total dairy.

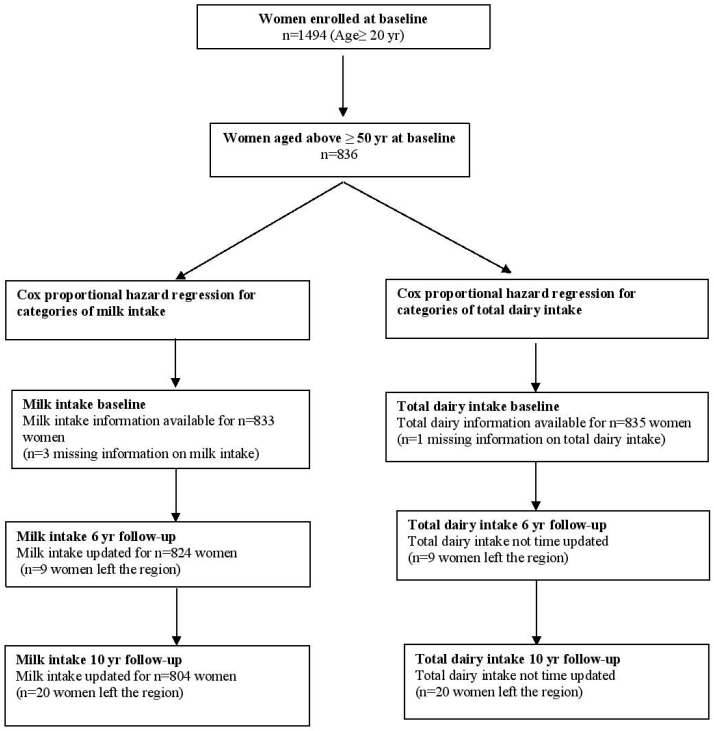

FIGURE 1
Participant flow chart. The figure represents the number of women at baseline, 6 and 10-year follow-up waves, and women left the region.

209x297mm (150 x 150 DPI)

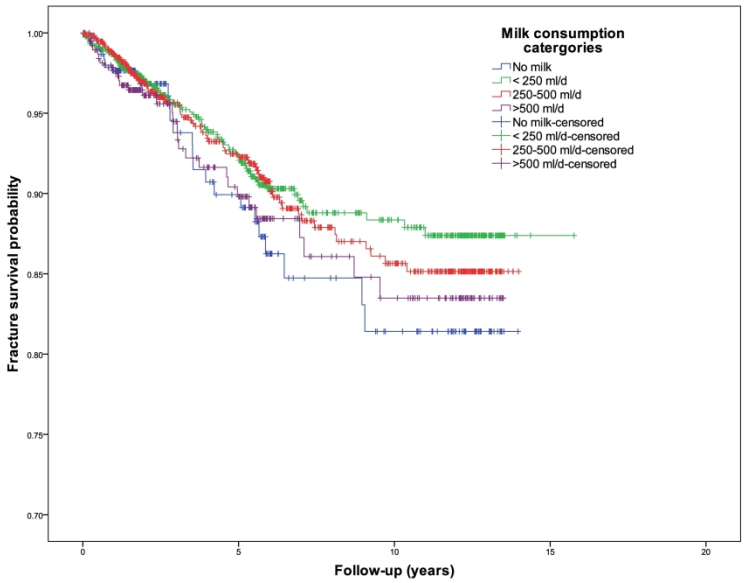

FIGURE 2
Kaplan-Meier survival plot for fractures in different milk consumption groups of women. The four curves represent fracture survival probability in different milk consumption groups (crude data). The lowest fracture survival probability is shown by the group consuming no milk.

352x211mm (300 x 300 DPI)

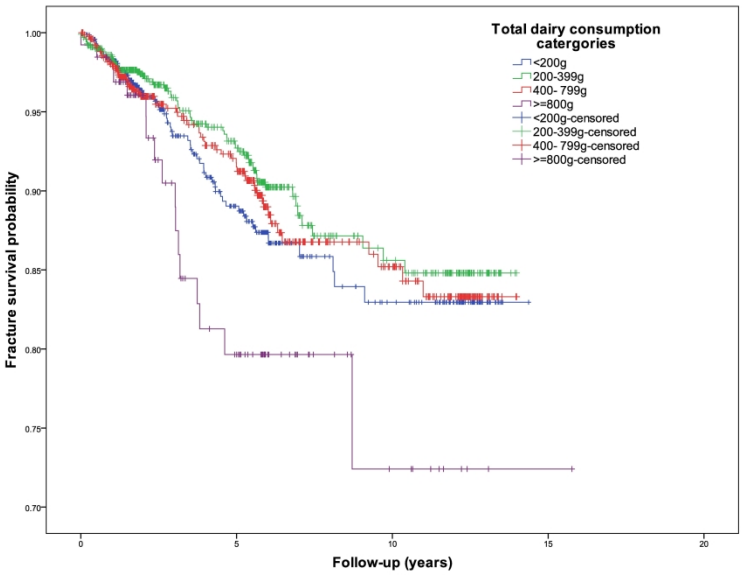

FIGURE 3

Kaplan-Meier survival plot for fractures in different total dairy consumption groups of women. The four curves represent fracture survival probability in different total dairy consumption groups (crude data). The lowest fracture survival probability is shown by the group consuming ≥800 g/d total dairy.

352x211mm (300 x 300 DPI)

Supplementary Table 1

Baseline characteristics of participants stratified by total dairy consumption categories[1]

	<200 g/d	200-399 g/d	400-799 g/d	≥ 800 g/d
Number of women	236	314	243	42
Age at entry, yr	69.1 (59.5-80.6)	70.4 (60.6-80.8)	69.5 (58.7-77.0)	72.5 (64.2-79.2)
Body mass index, kg/m^2	26.1 (23.2-29.7)	27.0 (23.7-30.4)	25.9 (23.6-29.7)	25.3 (23.5-27.1)
Milk, *n* (%)				
No milk	55 (26)	6 (2)	9 (4)	0 (0)
<250 mL/d	177 (76)	203 (65)	12 (5)	1 (2)
250-500 mL/d	2 (1)	105 (33)	178 (73)	1 (2)
>500 mL/d	0 (0)	0 (0)	44 (18)	40 (95)
Yogurt, g/d	0.0 (0.0-0.0)	0.0 (0.0-57.1)	28.6 (0.0-85.7)	85.7 (57.1-142.8)
Cheese, g/d	6.9(2.3-13.7)	9.1(4.6-16.0)	16.0 (8.9-31.4)	16.0 (8.0-35.4)
Ice-cream, g/d	0.0(0.0-7.7)	0.0(0.0-7.7)	1.0(0.0-11.6)	0.0 (0.0-7.7)
Bone mineral density, g/cm^2	0.809 ± 0.152	0.830 ± 0.162	0.843 ± 0.147	0.790 ± 0.132
Whole-body fat, kg	27.2 (20.3-33.1)	27.1 (20.3-33.1)	25.9 (20.8-32.7)	27.4 (27.4-27.4)
Lean mass, kg	36.8 ± 5.0	37.3 ± 4.4	37.4 ± 4.6	36.4 ± 4.0
Dietary calcium, *n* (%)				
<1000 mg/d	231(100)	310 (99)	157 (65)	0 (0)

≥1000 mg/d	0 (0)	3 (0.9)	86 (35.4)	41 (100)
Falls in the past, *n* (%)	43 (19)	68 (22)	49 (20)	11 (26)
Fractures in the past, *n* (%)	90 (38)	112 (36)	79 (33)	21 (50)
Incident cancer, *n* (%)	30 (13)	48 (15)	30 (12)	8 (19)
Diabetes, *n* (%)	17 (11)	25 (12)	23 (13)	4 (14)
Hypertension, *n* (%)	144 (61)	199 (63)	145 (60)	25 (60)
Smoking, *n* (%)				
Smokers	28 (13)	24 (8)	21 (9)	3 (7)
Non-smokers	208 (88)	290 (92)	222 (91)	39 (93)
Mobility, *n* (%)				
Highly active	109 (47)	152 (48)	134 (55)	20 (48)
Less active	125 (53)	162 (52)	109 (45)	22 (52)
Supplemental calcium, *n* (%)	34 (14)	40 (13)	30 (12)	12 (29)
Supplemental vitamin D, *n* (%)	30 (13)	37 (12)	23 (10)	10 (24)
Bisphosphonates, *n* (%)	1 (0.4)	3 (1)	4 (2)	0 (0)
Anabolic therapies, *n* (%)	0 (0)	0 (0)	0 (0)	0 (0)
HT, *n* (%)	37 (16)	49 (16)	36 (15)	4 (10)
IRSD *n* (%) / quintile				
1	37 (16)	71 (23)	42 (17)	5 (12)
2	48 (20)	73 (23)	48 (20)	12 (29)

3	64 (27)	61 (19)	57 (23)	10 (24)
4	39 (17)	55 (18)	36 (15)	9 (21)
5	48 (20)	54 (17)	60 (25)	6 (14)
Education, *n* (%)				
<12 years	205 (89)	275 (88)	207 (86)	35 (83)
≥12 years	26 (11)	37 (12)	34 (14)	7 (17)
Marital status, *n* (%)				
Living with partner	91 (39)	149 (48)	103 (42)	27 (64)
Living alone	143 (61)	165 (53)	140 (58)	15 (36)

[1]Data reported as mean± SD, median (IQR) or n (%); Total dairy comprises milk, cheese, yogurt and ice-cream

Reporting checklist for cohort study.

Based on the STROBE cohort guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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		Reporting Item	Page Number
Title and abstract			
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	1
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	#3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	#4	Present key elements of study design early in the paper	5
Setting	#5	Describe the setting, locations, and relevant dates, including periods of	5

		recruitment, exposure, follow-up, and data collection	
Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up.	5
Eligibility criteria	#6b	For matched studies, give matching criteria and number of exposed and unexposed	N/A
Variables	#7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6,7,8,9
Data sources / measurement	#8	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable.	6,7,8,9
Bias	#9	Describe any efforts to address potential sources of bias	16
Study size	#10	Explain how the study size was arrived at	17
Quantitative variables	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	10,11
Statistical methods	#12a	Describe all statistical methods, including those used to control for confounding	10,11
Statistical methods	#12b	Describe any methods used to examine subgroups and interactions	N/A
Statistical methods	#12c	Explain how missing data were addressed	6
Statistical methods	#12d	If applicable, explain how loss to follow-up was addressed	17
Statistical methods	#12e	Describe any sensitivity analyses	11
Results			
Participants	#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable.	Figure1

Participants	#13b	Give reasons for non-participation at each stage	Figure 1
Participants	#13c	Consider use of a flow diagram	Figure 1
Descriptive data	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable.	11/24
Descriptive data	#14b	Indicate number of participants with missing data for each variable of interest	N/A
Descriptive data	#14c	Summarise follow-up time (eg, average and total amount)	12/16
Outcome data	#15	Report numbers of outcome events or summary measures over time. Give information separately for exposed and unexposed groups if applicable.	
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	11/12
Main results	#16b	Report category boundaries when continuous variables were categorized	7
Main results	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	11
Discussion			
Key results	#18	Summarise key results with reference to study objectives	13
Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	17
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	18
Generalisability	#21	Discuss the generalisability (external validity) of the study results	18
Other Information			

Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	18